PB87-145678

Carcinogen-DNA Adducts: Introduction Literature Summary, and Recommendations

Lockheed Engineering and Management
Services Co., Inc., Las Vegas, NV

Prepared for

Environmental Monitoring Systems Lab.
Research Triangle Park, NC

Jan 87

U.S. Department of Commerce
National Technical Information Service
NTIS

PB87-145678

EPA/600/4-87/005
January 1987

CARCINOGEN-DNA ADDUCTS: INTRODUCTION, LITERATURE SUMMARY, AND RECOMMENDATIONS

by

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Contract No. 68-03-3249

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U.S. DEPARTMENT OF COMMERCE
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TECHNICAL REPORT DATA

(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/4-87/005	**2.**	**3. RECIPIENT'S ACCESSION NO.** PB87 145678/AS
4. TITLE AND SUBTITLE CARCINOGEN-DNA ADDUCTS: INTRODUCTION, LITERATURE SUMMARY, AND RECOMMENDATIONS		**5. REPORT DATE** January 1987
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) S. D. Soileau		**8. PERFORMING ORGANIZATION REPORT NO.**
9. PERFORMING ORGANIZATION NAME AND ADDRESS Environmental Programs Lockheed-EMSCO, Inc. Las Vegas, Nevada 89114		**10. PROGRAM ELEMENT NO.**
		11. CONTRACT/GRANT NO. 68-03-3249
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Monitoring Systems Laboratory-LV, NV Office of Research and Development U.S. Environmental Protection Agency Las Vegas, NV 89114		**13. TYPE OF REPORT AND PERIOD COVERED**
		14. SPONSORING AGENCY CODE EPA/600/07

15. SUPPLEMENTARY NOTES

16. ABSTRACT

This report summarizes the literature concerning adducts formed by xenobiotics with DNA and/or protein and discusses their feasibility as a monitoring tool for use in exposure and risk assessment. This report is divided into three segments. The first segment provides an introduction to DNA damage and its relation to carcinogenesis. This segment also discusses available methodology for the measurement of macromolecular (DNA, protein) adducts. The techniques were evaluated according to their sensitivity, selectivity, limitations and future possibilities. The next segment provides a summary of the current literature on the individual chemicals found to form adducts in both man and experimental animals. The information in this segment was also tabulated and is located in the appendix. Finally, the conclusion and recommendation section discusses the overall potential for the use of macromolecular adducts as a measure of dose, given the current technology. Recommendations on the analytical detection methodologies, applicable chemicals and populations to be used for a human monitoring pilot study were suggested.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	**19. SECURITY CLASS** *(This Report)* UNCLASSIFIED	**21. NO. OF PAGES** 134
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	**22. PRICE**

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

NOTICE

The information in this document has been funded wholly or in part by the U.S. Environmental Protection Agency under contract number 68-03-3249 to Lockheed Engineering and Management Services Company, Inc. It has been subject to the Agency's peer and administrative review, and it has been approved for publication as an Agency document.

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ABSTRACT

This report summarizes the literature concerning adducts formed by xenobiotics with DNA and protein in order to determine their feasibility as a monitoring tool for use in exposure and risk assessment and to propose compounds and methods that may be appropriate for preliminary field studies. This report is divided into three segments.

The first segment provides an introduction to DNA damage and its relation to carcinogenesis. This segment also discusses available methodology for the measurement of macromolecular (DNA, protein) adducts. The techniques were evaluated according to their sensitivity, selectivity, limitations, and future possibilities. The next segment provides a summary of the current literature on the individual chemicals found to form adducts in both man and in experimental animals. The information in this segment and additional information was tabulated and is presented in the appendix. Finally, the conclusion and recommendation section discusses the overall potential for the use of macromolecular adducts as a measure of dose, given the current technology. Recommendations on the analytical detection methodologies, applicable chemicals, and populations to be used for a human monitoring pilot study were offered.

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INTRODUCTION

The Environmental Protection Agency is charged to protect human health and the environment, and it has acted by placing restrictions and regulations on chemicals that have been shown to be detrimental to human health or to the environment. Accurate dose measurements are critical in the evaluation of health risks and in the development of regulations that may be needed for protection from chemicals released into the environment.

In the past, human exposure to xenobiotics has been estimated by direct measurement of the concentration or amount of the xenobiotic present in one or more environmental compartments (e.g., air, water, foodstuffs, etc.). Such data can only give crude estimates of the dose received because additional information is required to estimate more accurately the dose (e.g., duration of exposure, pulmonary ventilation, food consumption, etc.).

Biological monitoring is the measurement of the concentration of xenobiotics in organisms (e.g., man). Examples of biological monitoring would include measurement of xenobiotics or their metabolites in blood or urine or measurement of reaction products between the compounds and cellular macromolecules such as proteins and DNA. Biological monitoring gives a better estimate of the dose received because it corrects for interindividual variations in absorption, metabolism, and excretion. This kind of chemical dosimetry also integrates exposure from all sources, and therefore can be used as a basis for the estimation of the total potential risks from multiple chemicals. Because of these correcting factors, biomonitoring is related more directly than environmental measurement to the adverse effects induced by xenobiotics (41).

The Environmental Protection Agency has developed an initiative designed to develop, refine, and apply appropriate biomarkers that can be used in conjunction with other environmental monitoring data to provide a better estimate of risk to individuals and populations. By linking biological measurements to environmental monitoring measurements, it will be possible to determine relationships that exist between total exposure, dose, and disease.

The first stage of the EPA initiative is to evaluate the feasibility of using biomarkers as a monitoring tool for use in exposure and risk assessment. This will include a compilation of available biomonitoring methods for assessing environmental exposures and of methods for predicting associated health risks. The purpose of this document is to summarize the literature concerning adducts formed by xenobiotics with DNA and protein in order to determine their feasibility for use in exposure and risk assessment and to propose compounds and methods that may be appropriate for preliminary field studies. This report is divided into three segments.

The first segment provides an introduction to DNA damage and its relation to carcinogenesis. This segment also discusses available methodology for the measurement of macromolecular (DNA, protein) adducts. The techniques were evaluated according to their sensitivity, selectivity, limitations, and future possibilities. The next segment provides a summary of the current literature on the individual chemicals found to form adducts in both man and experimental animals. The information in this segment was also tabulated and is located in the appendix. Finally, the conclusion and recommendation section discusses the overall potential for the use of macromolecular adducts as a measure of dose, given the current technology. Recommendations on the analytical detection methodologies, applicable chemicals, and populations to be used for a human monitoring pilot study were suggested.

Chemical Carcinogenesis

The ability of chemicals to induce cancer has been known for more than two centuries. It was observed in 1776 by Sir Percival Pott that chimney sweeps developed scrotal cancer and that the cancer was associated with exposure to soot and tars. Initially, it was believed that chronic irritation was the cause of cancer, but this theory could not explain how short exposures to chemicals were sufficient to induce cancer and that cancer could appear many years after exposure to carcinogenic chemicals. Many chemicals were found to be carcinogenic, but there appeared to be no correlation between chemical structure and the ability of a particular chemical to induce cancer. Researchers found that the metabolites of some carcinogens were more active than the parent compounds themselves. They then looked at the compounds and their active metabolites to determine if similarities existed in their chemical activities. It was discovered that the great majority of active compounds contained an electrophillic group (i.e., an atom possessing a low electron density). These electron-poor sites attack sites of high electron density. Figure 1 shows sites of interaction of chemical carcinogens with DNA *in vivo* and *in vitro*. Nitrogen and oxygen atoms in proteins are also susceptible to attack.

These electrophillic compounds can be divided into two classes; direct acting and indirect acting carcinogens. Direct acting compounds possess strong electrophillic sites and can covalently bind to DNA without chemical modification. Nitroso compounds are an example of direct acting carcinogens. Figure 2 shows other carcinogens of this class. Indirect acting carcinogens cannot alkylate DNA directly; they must be metabolically activated to form an electrophillic species before alkylation can occur. Benzo(a)pyrene is an example of an indirect acting carcinogen. It must be metabolically activated to form the diol-epoxide metabolite before alkylation can occur. Figure 3 shows some other carcinogens of this class. It was postulated that the compounds of interest may damage DNA and that this damage was the first step in chemical carcinogenesis. It was also postulated that this damage must be inherited by daughter cells, so the damage must induce a change in the DNA that can be passed on to future generations of cells. In short, a mutational change in the DNA sequence within a gene must be induced in the affected cell. Mutation induced by DNA alkylation is postulated to occur by the following mechanism. The active form of the carcinogen alkylates DNA in such a way as to alter the manner in which it base pairs during replication. An example would be the base pairing of O^6-methyl guanine. Guanine usually base pairs with cytosine (Figure 4). However, if guanine is methylated at the O6 position, its ability to hydrogen bond is altered to the extent that it prefers to base pair with thymine. When replication of the single strand of DNA containing the methylated guanine occurs, a thymine will replace cytosine at the position of the alkylated guanine (Figure 4). Therefore, alkylation of bases in DNA can induce the formation of stable mutations. These changes in the DNA sequence can result in failure to transmit genetic information accurately.

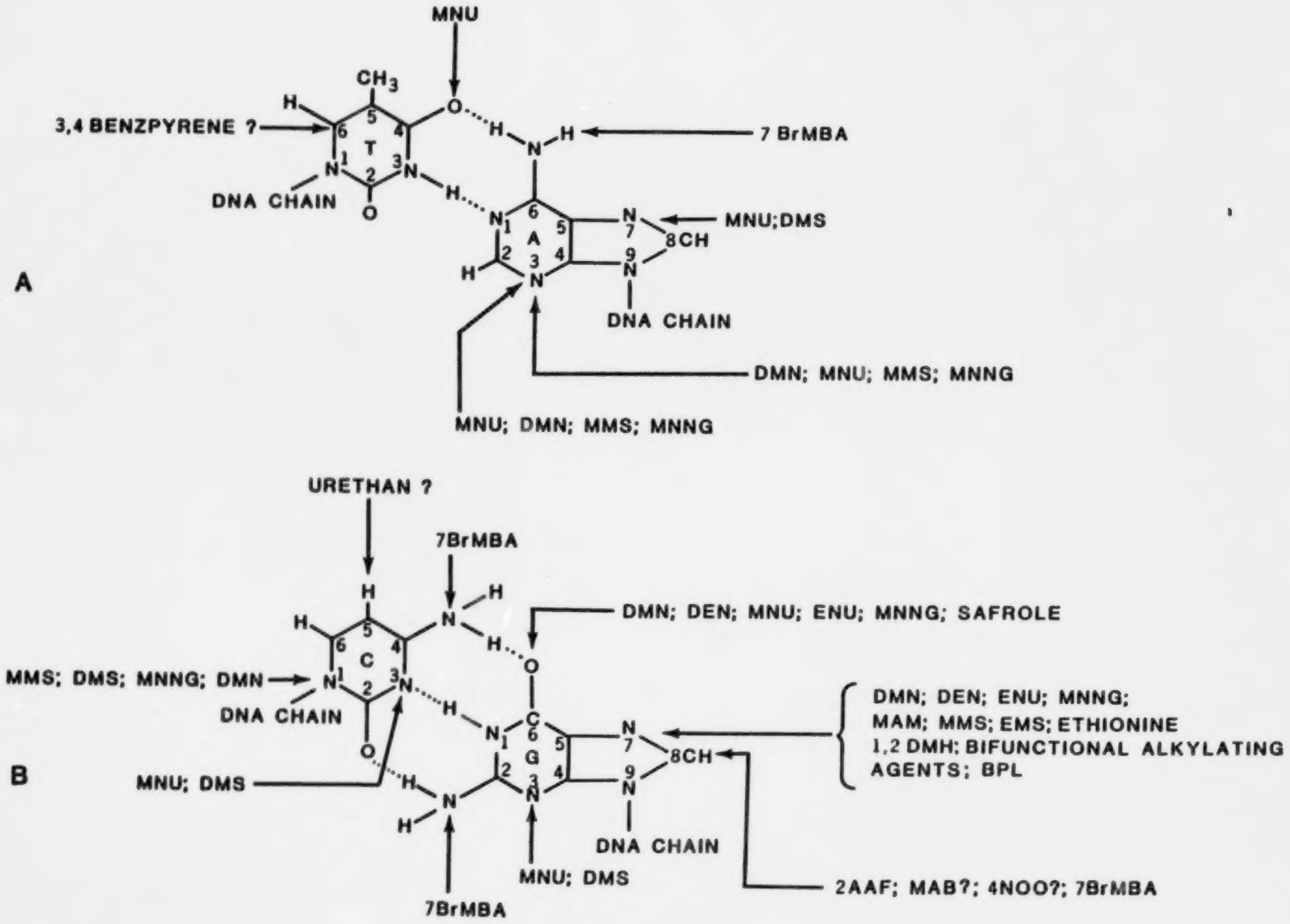

A
MNU
CH3
3,4 BENZPYRENE ?
7 BrMBA
T
DNA CHAIN
MNU;DMS
8CH
DNA CHAIN
DMN; MNU; MMS; MNNG
MNU; DMN; MMS; MNNG
B
URETHAN ?
7BrMBA
DMN; DEN; MNU; ENU; MNNG; SAFROLE
MMS; DMS; MNNG; DMN
C
DNA CHAIN
DMN; DEN; ENU; MNNG;
MAM; MMS; EMS; ETHIONINE
1,2 DMH; BIFUNCTIONAL ALKYLATING
AGENTS; BPL
G
8CH
MNU; DMS
DNA CHAIN
MNU; DMS
7BrMBA
2AAF; MAB?; 4NOO?; 7BrMBA

Figure 1. Sites of interaction of chemical carcinogens with DNA
in vivo (A) and *in vitro* (B). Adapted from (188a).

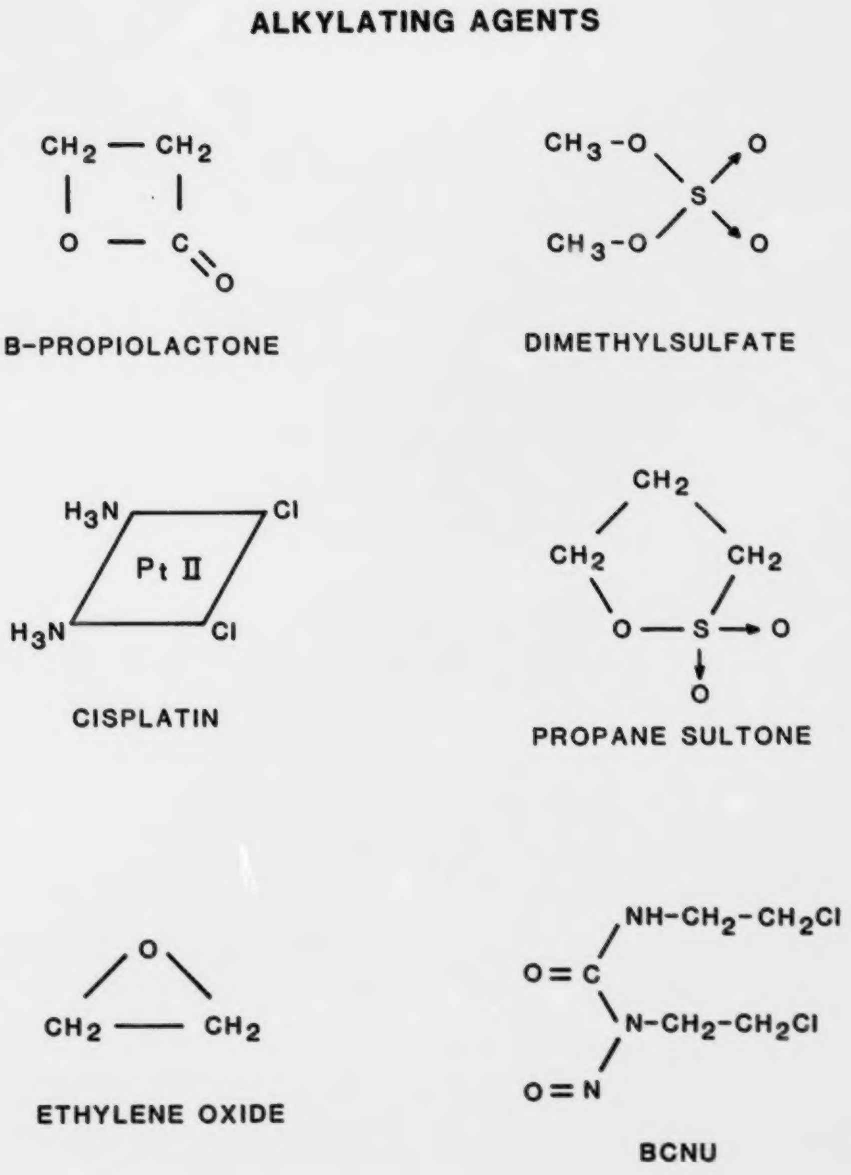
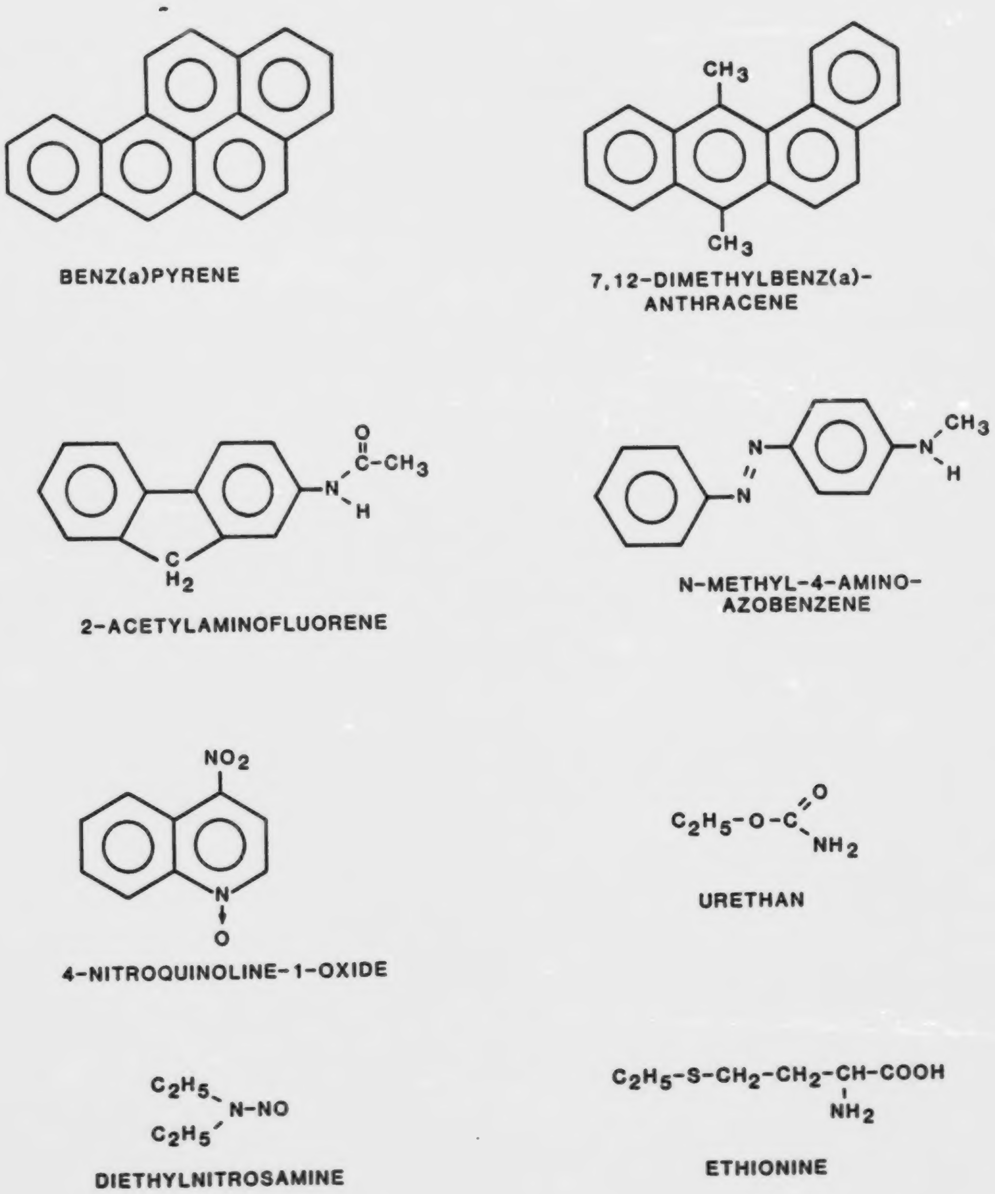

ALKYLATING AGENTS
B-PROPIOLACTONE
DIMETHYLSULFATE
CISPLATIN
PROPANE SULTONE
ETHYLENE OXIDE
BCNU

Figure 2. Examples of direct-acting carcinogens. Adapted from (188a).


CH3
CH3
O
C-CH3
N
H
C
H2
N
N
N
CH3
H
NO2
N
O

BENZ(a)PYRENE

7,12-DIMETHYLBENZ(a)-ANTHRACENE

2-ACETYLAMINOFLUORENE

N-METHYL-4-AMINO-AZOBENZENE

4-NITROQUINOLINE-1-OXIDE

$C_2H_5-O-C(=O)-NH_2$

URETHAN

$(C_2H_5)_2N-NO$

DIETHYLNITROSAMINE

$C_2H_5-S-CH_2-CH_2-CH(NH_2)-COOH$

ETHIONINE

Figure 3 Examples of indirect-acting carcinogens. Adapted from (188a).

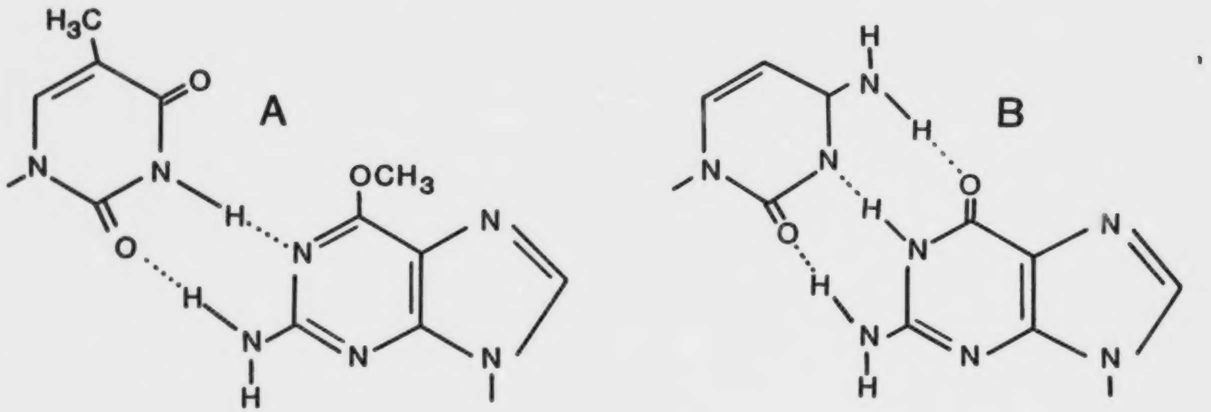


Figure 4. Mispairing induced by O^6-methylguanine.

A. O^6-Methylguanine-thymine base pair.
B. Normal G-C base pair. Adapted from (188a).

However, genotoxic damage, in and of itself, is not sufficient to induce tumor formation. In 1941, Rous and Kidd first suggested a two stage mechanism for the development of cancer: an initiation step followed by promotion with another agent (186). Initiation is essentially the damage of cellular DNA that results in DNA miscoding. The term promotion is used to designate the process by which initiated cells are encouraged or accelerated to become neoplastic (i.e., cancerous). This hypothesis was extended by Berenblum and Shubik in the late 1940's (36). The investigators determined that neither methylcholanthrene (the initiator) nor the croton oil (the promotor) produced tumors when applied separately. However, when the promoter was applied after application of the initiator, tumor development was noted (Figure 5).

Animal experiments indicate that initiation is an irreversible event, whereas promotion is reversible. Application of a promotor to an experimental animal that was treated with an initiator a year previously still resulted in skin tumor formation (220). However, if application of a promotor is discontinued before the cells are transformed to a neoplastic state, tumor development is not seen.

Genotoxic Damage and its Relationship to Carcinogenesis/Mutagenesis

Although initiation implies DNA damage, not all DNA damage can be termed initiation as such. If DNA damage is very severe, the cell is unable to produce essential proteins and the cell dies. Sister chromatid exchange (SCE) is a type of chromosomal damage that is characterized by the transfer of chromatin between two chromatids of a chromosome. Although cigarette smoke condensate can induce a concentration-dependent increase in the frequency of SCE's in human lymphocytes (95), SCE's appear to correlate better with cell death than with cell mutation rates (36,152). DNA damage can also be expressed as actual breaks in the DNA strand. Another gross change that has been used as a method of detecting DNA damage is the measurement of micronuclei in maturing erythrocytes and in lymphocytes. Colchicine, a drug that inhibits mitotic spindle formation, can induce micronuclei formation in dividing cells. Unscheduled DNA synthesis (UDS) is also a measure of DNA damage. UDS is a measure of excision repair of damaged DNA. This means that damaged DNA is removed enzymatically and is replaced with undamaged nucleotides.

Although a mutation is postulated to be required in the multistage process of cancer induction, not all mutations will lead to the induction of cancer. For example, a mutation may lead to the change in a single amino acid in a particular protein or enzyme. This may or may not be fatal, but it is not a cancer initiation step. Congenital enzyme deficiencies, such as Phenylketonuria (PKU), or Sickle Cell Anemia are examples of this type of mutation. A mutation may occur such that an amino acid codon is converted to a stop codon. This type of mutation is usually fatal. Bifunctional alkylating agents can cause crosslinking of double-stranded DNA and can prevent complete DNA replication and cause cell death if not repaired. Many cancer chemotherapeutic agents, such as cisplatin, cause cell death via such a mechanism. It is not known how mutations cause normal cells to become neoplastic, but there is evidence that some mutations activate certain genes that can transform a cell to a neoplastic state (205,210). These genes are referred to as oncogenes. Experimental evidence indicates that chemical carcinogens, in concert with certain viruses, can trigger the neoplastic transformation of human epithelial cells (183a). The oncogenes may be present

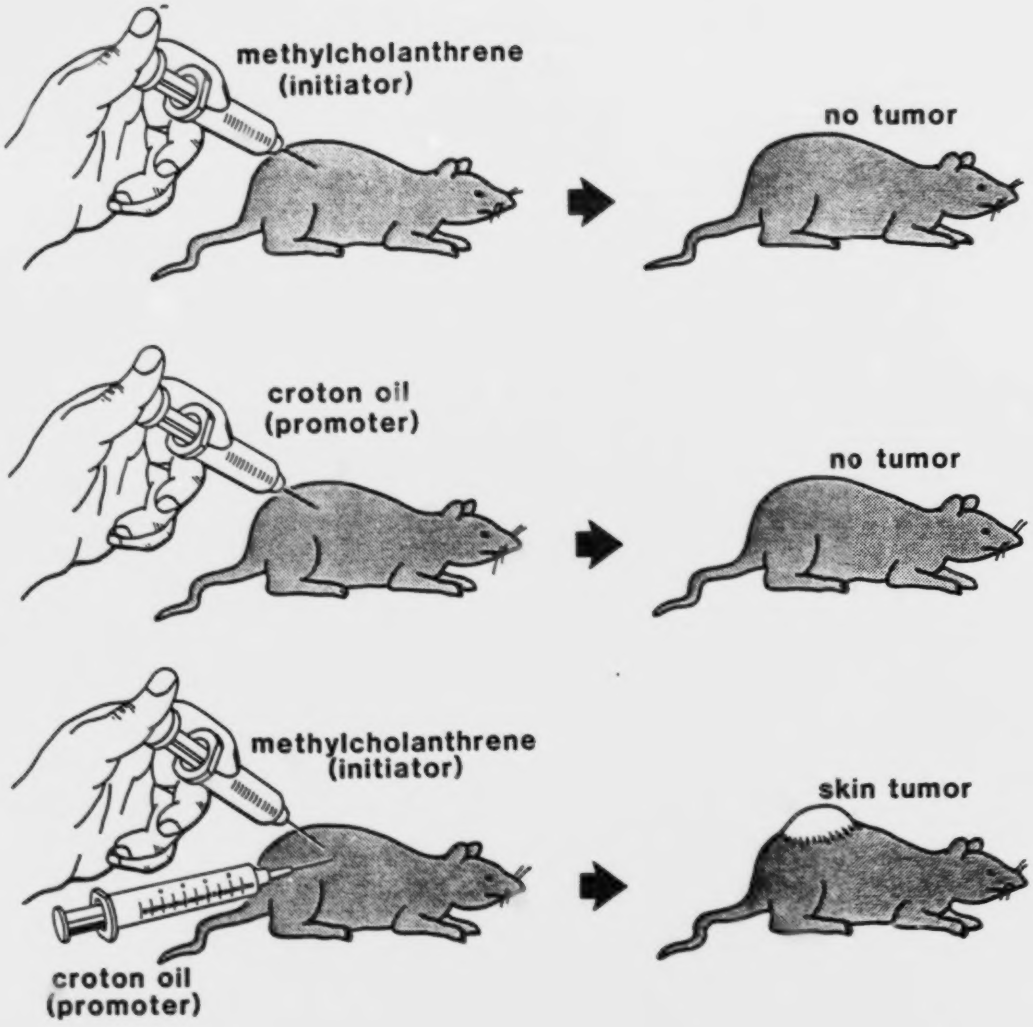

methylcholanthrene
(initiator)
no tumor
croton oil
(promoter)
no tumor
methylcholanthrene
(initiator)
skin tumor
croton oil
(promoter)

Figure 5. Relationship between initiation, promotion, and tumor formation. Adapted from (120).

in the cellular genome, or they may be introduced into the cell by means of a virus. In summary, very specific mutations are required for neoplastic conversions to occur. Although very specific mutations are required for neoplastic conversion, non neoplastic-inducing mutations also take place. Biological methods exist for both in vivo and in vitro measurement of the induction of mutations. The Ames test measures the ability of a chemical to induce a specific mutation in the his^- Salmonella bacteria to allow the mutant bacteria to synthesize histidine. Other assays measure hypoxanthine guanine phosphoribosyl transferase (HGPRT) and hemoglobin mutations in blood cells.

It is believed that the primary mechanism of chemically-induced mutation is through miscoding induced by carcinogen-DNA adducts. The existence of DNA adducts was first shown in 1962; a specific alkylated base, 7-methylguanine, was isolated from cells treated with dimethylnitrosamine (137). Even though this adduct is now considered not to be important in the mechanism of carcinogenicity of alkylating agents, this study was important in that it stimulated a large number of studies in the area of carcinogen-DNA adducts.

Many studies have shown that carcinogen-DNA adduct levels are correlated with the frequency of mutagenic/carcinogenic alterations. Alkylating agents can attack nitrogen and oxygen atoms in purines and pyrimidines in DNA, and in many instances, a relationship was found to exist between the levels of O^6-alkylguanine and the frequency of tumor occurrence (148, 208, 222). Most other carcinogens show a similar correlation. 2-Napthylamine (2-NA) is a urinary bladder carcinogen but not a liver carcinogen in dogs. The binding of radiolabelled 2-NA to DNA in dog bladder and liver was measured in a recent study. Eight days after administration of 2-NA, total binding to DNA was eight times higher in the bladder than in the liver (106). In another study 1-napthylamine (1-NA) and 2-NA were fed to Sprague-Dawley rats. 1-NA was found to be more carcinogenic than 2-NA, and this difference was correlated with the greater binding (about 20 fold) of 1-NA to DNA (60).

Unfortunately, the relationship between DNA adduct levels and the frequency of tumor formation is rarely a simple direct correlation. For example, when mice were treated with the carcinogen 15, 16-dihydro-11-methylcyclopenta[a]phenanthrene, initial DNA levels in mouse liver were twice as high as were found in skin and lung DNA (2). However, this compound is carcinogenic in mouse lung and skin but not in the liver. When the persistence of the adducts was measured, it was found that although liver DNA contained higher initial levels of DNA adducts, repair of the damage occurred at a faster rate than in mouse lung or skin (2). It therefore appears that the persistence of DNA adducts is an important factor in relating DNA adduct levels to tumor frequency. In several other studies, the same inverse correlation was found between DNA repair of chemically induced lesions and the frequency of mutagenic or carcinogenic events. 7, 12-Dimethylbenz[a]-anthracene, a potent mammary carcinogen in certain strains of rats, was administered to a resistant strain of rat, the Long-Evans rat, and to a susceptible strain, the Sprague-Dawley rat (50). Initial DNA adduct levels were significantly higher in the resistant strain. However, the resistant strain showed significant DNA repair 14 days after carcinogen administration, whereas the susceptible strain showed no significant loss of DNA adducts. Using genetic engineering techniques, a tetranucleotide containing O^6-methylguanine was spliced into an E. coli bacteriophage. The virus (bacteriophage) was allowed to infect cells with either a normal or deficient DNA repair mechanism. Because viruses use host cell enzymes for their own replication, one would expect to find an increased mutation frequency in the progeny virus from the repair-deficient cells. An increased mutation frequency was found; in fact, the mutation frequency was 50 times higher in the repair-deficient progeny phage (62). A study that measured the formation of aflatoxin B_1 in rat liver showed that the initial principal adduct was removed with a halflife of 6.5 hours. However, a small percentage (20 percent) of the adducted guanine residues underwent an imidazole ring scission, and these ring-opened adducts were not rapidly repaired. If multiple doses were administered in a regimen shown to produce a high incidence of hepatocellular carcinoma, a time-dependent increase in the imidazole ring-opened adduct was detected (48). Similarly, the persistence of 3-methylcholanthrene (3-MC) binding in mouse liver and lung DNA was measured for up to 28 days after administration of a single i.v. dose. Mouse lung is susceptible to 3-MC (in terms of carcinogenicity), whereas mouse liver is resistant. Both tissues showed a decrease in adduct levels 28 days after administration of 3-MC; however, adduct levels were still measurable in lung DNA, whereas adduct levels in the liver were no longer measurable (61a).

Another factor that must be considered when relating adduct levels to carcinogenesis is the rate of cell division of the damaged cell. Unless the damaged nucleotides are 'fixed' by miscoding during replication before DNA repair occurs, a mutational event will not occur. The relatively high rate of cell division in mouse lung and skin cells may partially explain why such cells are susceptible to 15, 16-dihydro-11-methylcyclopenta[a]phenanthrene-17-one, even though initial adduct levels in the liver are twice as high (62). The rate of cell division in the liver is normally quite low. A recent report by Swenberg et al. (208) proposed that all promutagenic DNA adducts are important in the mechanism of carcinogenesis, and the extent of cell replication for each population of cells exposed influences the probability of tumor formation.

These three factors (adduct levels, adduct repair, and rate of cell division) are important in relating DNA adducts to carcinogenesis, but other unknown factors are probably important. DNA adducts were found to be persistent in rat liver not only for two hepatocarcinogens, N-hydroxy-2-acetylaminofluorene (N-OH-AAF) and N-hydroxy-4-acetylaminobiphenyl, but also for the non-hepatocarcinogen, N-hydroxy-2-acetylaminophenanthrene (75). In another study, persistence of DNA adducts in a non-target tissue (kidney) was noted after multiple treatments with N-OH-AAF (31). In summary, although a correlation has been noted between certain DNA adducts and tumor formation, research is still needed to elucidate all of the mechanisms involved in the carcinogenic process.

As has been discussed earlier, exposure assessment is accomplished at present by monitoring the environment for carcinogens and also by monitoring biological fluids or tissues for the same. Monitoring biological fluids or tissues for carcinogens is more accurate than environmental data because it factors in the effects of variation in absorption and metabolism of the the compounds. However, measurement of free carcinogen concentrations does not measure actual DNA damage or related phenomena (i.e., protein adduct formation). For example, interindividual variations in binding of benzo[a]pyrene to DNA is about 50- to 200-fold (218, 78). Other compounds show at least 10-fold variation among individuals (218). Measurement of damage in the organ that is susceptible to the carcinogen (target site) would provide a more accurate estimate of the risk (17). Carcinogen adducts would provide such a measure of the dose received. DNA adducts give a measure of genotoxic exposure, because the levels measured are the net of the adducts formed minus adducts lost through enzymatic or non-enzymatic repair,or both. Protein adducts, on the other hand, are stable over the life span of the protein and as such provide an integration of exposure over the life of the protein (235). Because of these properties, measurement of DNA adduct levels could be more useful in risk assessment, and measurement of protein adduct levels could be more useful in exposure assessment.

Biological Markers for DNA Damage

When an organism is exposed to a genotoxic agent, many sites are attacked. DNA of both susceptible and resistant cell types are attacked, and other molecules containing nucleophillic sites, such as proteins and RNA, can also be modified by the same electrophillic agents. Because of the non-specific action of electrophiles, there are many types of molecules that can be used as molecular dosimeters for genotoxic damage.

The ideal dosimeter for relating dose to biological effect is the DNA from the cell population(s) that is susceptible to the particular carcinogen being studied. Different cell types have different levels of xenobiotic-metabolizing enzymes and DNA repair enzymes, and physical properties of the carcinogen may result in different levels of the carcinogen in different cell types. There are many steps between exposure to a carcinogen and the induction of target cell DNA damage (Figure 6). Small changes in any or all of these steps can lead to great changes in the levels of DNA adducts. For these reasons, measuring DNA adduct levels in the target tissue would give the best possible correlation between environmental levels of the carcinogen and target site damage, as well as the best possible correlation between target site damage and tumor formation. Unfortunately, there is an important disadvantage to using target site DNA as a molecular dosimeter: accessibility. It is extremely difficult to obtain a sample; a biopsy, at a minimum, is usually required. Samples of this type are usually obtained during elective surgery or during an autopsy conducted immediately after death. Even though difficult to obtain, such samples can be of some use. For example, benzo[a]pyrene-DNA adducts were detected in cultured human colon of persons with and without colon cancer (13). The use of this sample in an epidemiology study with a large number of participants is essentially impossible.

The problem of availability can be averted by measuring DNA adduct levels in tissues and fluids that are easily accessible and that can be obtained with a minimum of invasiveness. Circulating DNA, in the form of white blood cell (WBC) DNA, is readily available and would be amenable for use in a large epidemiology study. The main disadvantage of this sample type is that the levels of DNA adducts found in WBC's may not relate to the levels of DNA adducts at the target site.

DNA damage can also be measured indirectly by measuring DNA adducts that have been excreted in urine. The detection of DNA adducts in urine would theoretically give an indication of recent exposure to certain compounds. In addition, if information were available on the dose received, such measurements would indicate the level of DNA repair in the individual (235). For a given dose received, the more rapid the repair, the more adducts would be detected in the urine. This information would complement DNA adduct level data measured in either the target or circulating DNA. Aflatoxin B1-DNA

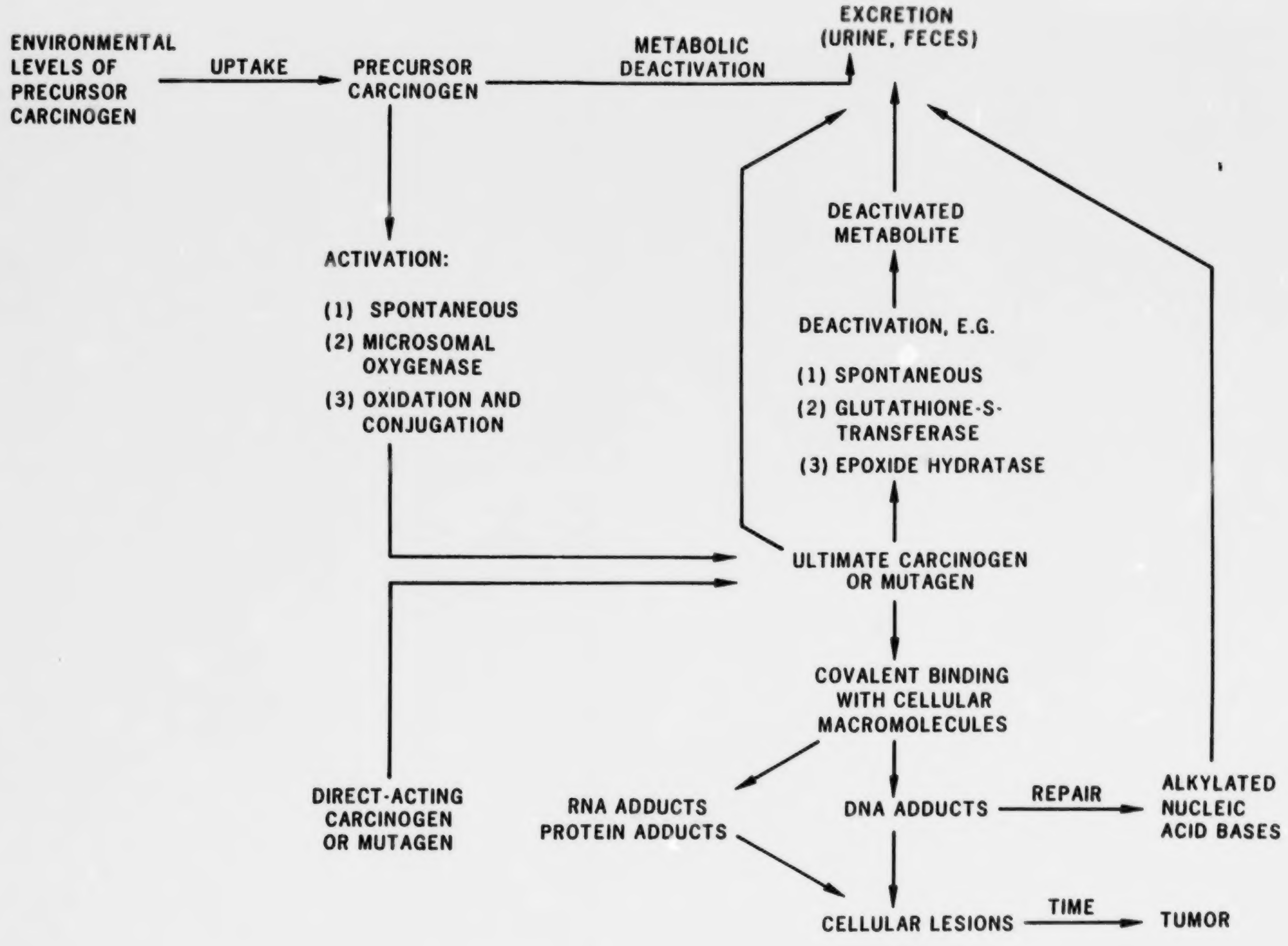

ENVIRONMENTAL LEVELS OF PRECURSOR CARCINOGEN
UPTAKE
PRECURSOR CARCINOGEN
METABOLIC DEACTIVATION
EXCRETION (URINE, FECES)
ACTIVATION:
(1) SPONTANEOUS
(2) MICROSOMAL OXYGENASE
(3) OXIDATION AND CONJUGATION
DEACTIVATED METABOLITE
DEACTIVATION, E.G.
(1) SPONTANEOUS
(2) GLUTATHIONE-S-TRANSFERASE
(3) EPOXIDE HYDRATASE
ULTIMATE CARCINOGEN OR MUTAGEN
COVALENT BINDING WITH CELLULAR MACROMOLECULES
DIRECT-ACTING CARCINOGEN OR MUTAGEN
RNA ADDUCTS
PROTEIN ADDUCTS
DNA ADDUCTS
REPAIR
ALKYLATED NUCLEIC ACID BASES
CELLULAR LESIONS
TIME
TUMOR

Figure 6 Relationship between exposure, dose, and health effects. Adapted from (220a)

adduct excretion has been monitored by using this strategy (15, 35). The disadvantage of this method is that measurement of excreted DNA adducts gives no indication as to the cell population(s) that contained the adducts. For this source to be a useful indicator of dose, studies would have to be conducted to understand the relationship between adduct levels in the urine and adduct levels in the target cell DNA.

If one wants to determine DNA adduct structure or if one wants to study reaction mechanisms of the compounds with DNA, in vitro studies can be of some use. The compounds of interest are reacted with DNA (usually calf thymus DNA) and isolation, characterization, and quantitation of the carcinogen-DNA adducts are performed in a manner similar to that used in in vivo studies. Several compounds have been studied in this manner, including derivatives of benzo[a]pyrene (72), azo dye derivatives (135, 213) and 15, 16-dihydro-11-methylcyclo-penta[a]phenanthren-17-one (47). This procedure offers the advantage of greatly increasing the levels of adducts in the DNA, thus facilitating identification of adduct structure. If the carcinogen is direct acting, that is, it requires no metabolic activation, the compound is simply mixed with the DNA in an aqueous solution. If the compound requires metabolic activation, either an enzymatic metabolizing system must be included (47), or a stable derivative of the electrophilic compound must be synthesized (22, 135, 213). One disadvantage of this method is that the adducts formed may not be the same as the ones formed in vivo (22).

Although measurements of DNA adducts are an ideal measurement when one wants to relate environmental levels to biological endpoints, they are not necessarily an ideal measure of dose. The levels of DNA adducts are modulated by enzymatic and nonenzymatic repair, and cell division can dilute the concentration of the adducts. As was stated earlier, electrophillic compounds also form covalent adducts with proteins. Carcinogen-protein adducts are usually stable over the life of the protein and as such make a good integrator of exposure during this time period. Most of the early studies have measured binding to proteins by using radiolabelled carcinogens, although GC-MS or amino acid sequencing techniques have also been used (64, 99; Table 1). These adducts should be easily amenable to immunological methods of detection, and detection levels should be very low. Hemoglobin has received the most attention in this area, presumably because it is easily accessible and because it is possible to obtain large quantities of the protein. The lifespan of an erythrocyte is about 120 days, so measurement of hemoglobin adducts integrates exposure over months. Human serum albumin has a half life of about 20 days (232). Protein adducts measured on serum albumin would integrate exposure over a much shorter time span. Protein adduct formation shows a linear dose-response curve for most compounds (20, 64, 99, 155, 164, 193, 212), and protein adduct formation tends to correlate with DNA adduct formation in various tissues. If one wanted to correlate a particular DNA adduct formation with protein adduct formation, the appropriate animal experiments would have to be conducted. Because the levels of adducts that would be detected in the general population would be very low, one would have to make sure that the dose response relationship was linear at very low carcinogen concentrations. One would not expect the correlation between DNA and protein adduct levels to be perfect in continuous dosing regimen because of the occurrence of DNA repair, cell replication, etc. Also, the toxicokinetics of the carcinogen may also affect DNA adduct formation in a different manner.

TABLE 1. PROTEIN BINDING OF ALKYLATING AGENTS

COMPOUND	PROTEIN	SPECIES
Methyl methanesulfonate	Hb	mouse
	Hb	rat
N-Nitrosodimethylamine	Hb	mouse
	Serum	rat
	Hb	rat
	Erythrocyte	human
Methyl bromide	Hb	mouse
Methyl chloride	Erythrocyte	human
	plasma	human
Dichlorvos	Hb	mouse
Ethylene oxide	Hb	mouse
	Hb	human
	Hb	rat
Propylene oxide	Hb	rat
Vinyl chloride	Hb	mouse
Ethylene	Hb	mouse
Benzo[a]pyrene	Hb	mouse
	Hb	rat
Chloroform	Hb	rat
	Hb	mouse
2-Acetylaminofluorene	Hb	rat
	Hb	mouse
	Serum	rat
Benzyl chloride	Hb	mouse
Aflatoxin B1	Hb	rat
trans-Dimethylaminostilbene	Hb	rat
	Plasma	rat
trans-4-Aminostilbene	Hb	rat
	Plasma	rat
4-Aminobiphenyl	Hb	rat
	Albumin	rat

Hb = Hemoglobin
NOTE: Adapted from (64)

Although it is possible to measure both DNA and protein adducts in humans, it will likely be more difficult to relate DNA adduct levels to environmental levels. As can be seen in Figure 6, many factors influence the level of an electrophillic compound in an organism (or humans). If one wants to relate environmental levels of carcinogens to levels in the body, one has to correct for interindividual variation. A direct or indirect approach can be used for this correction. In the direct approach, one could try to define all of the factors that influence levels of a compound in the body and then try to quantitate each factor for each individual in the study. This would involve, at a minimum, measurement of various enzyme levels. Even if all factors could be measured quantitatively, this model would assume that enzyme levels remain constant. This is certainly not the case. Additional animal experimentation would be required for this approach to be viable. One idea that might be investigated is to determine if the repair capability of an individual can be estimated by determining the ratio of DNA or protein adducts in the measured tissue or fluid to the levels of DNA adducts in the urine. It might also be helpful to measure levels of metabolites in the urine. This would given an indication as to how much detoxification of electrophillic species occurred. In the indirect approach, a large enough sample size is chosen such that interindividual variation tends to cancel out. Another problem that must be faced is the randomness of dosing in the real world. In the general population, dosing would not occur as a single dose of a carcinogen; rather, small doses of many carcinogens would occur at irregular time intervals. It is likely that many preliminary animal experiments would be required in order to understand human dose-response relationships in the general population. Even though much research still needs to be conducted, the measurement of adduct levels in individuals would provide a much better estimate of dose than the measurement of environmental levels of carcinogens.

TABLE 2. ASSAYS FOR THE DETECTION OF CARCINOGEN-DNA ADDUCTS

METHOD	Limit of detection fmole	Amount of DNA used per analysis mg*[a]	Modificatio analyzed DN adducts/base
UV in line with HPLC [major benzo(a)pyrene-DNA adduct[b]]	100,000	2600	2×10^{-5}
Fluorescence in line with HPLC (BPDE-I-tetrol)	31	100	1×10^{-7}
Photon counting synchronous scanning fluorimetry			1×10^{-6}
Immunoassays			
Polyclonal rabbit antibodies against BPDE-I-DNA			
Competitive assays			
RIA	5,300	1	1.7×10^{-3}
ELISA	55	1	1.8×10^{-5}
USERIA	12	1	3.9×10^{-6}
USERIA	10	25	1.4×10^{-7}
Noncompetitive assays			
USERIA	3	0.01	9.7×10^{-5}
Monoclonal antibodies against BPDE-I-DNA			
Competitive ELISA	19	0.005	1.2×10^{-3}
Noncompetitive ELISA	3	0.0002	4.9×10^{-3}
^{32}P-postlabeling	0.03-0.3	1	$1 \times 10^{-7}-10^{-8}$

[a] As a guideline, 10-100 mg DNA is recoverable from 0.2-1 g tissue or the buffy coat of 25-50 ml human blood. Adapted from (235).

[b] Benzo(a)pyrene is used as an example in this table since these techniques have been applied to the detection of its major adduct, 10-(deoxyguanosin-N^2-yl)-7,8,9-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene.

METHODS FOR DETECTING CARCINOGEN-DNA ADDUCTS

During the past 10 years, many different methods have been developed to measure DNA-carcinogen adducts. The methods have widely varying sensitivities and varying levels of applicability. Table 2 lists available methods, sensitivities, and amount of DNA required for analysis. In the discussion of each of the methods, isolation of the DNA from the selected sample is required prior to the use of the detection methods.

Most of the work in DNA adduct research in animal models has been accomplished using radiolabelled carcinogens and subsequent measurement of the radioactivity via scintillation counting. The primary isotopes used are ^{14}C and ^{3}H. The advantage of ^{3}H as the isotope is that it is usually easily incorporated into compounds and can be obtained at a relatively high specific activity. The use of a high specific activity radioisotope increases the sensitivity of the compound. Using ^{3}H, adducts can be detected down to a level of a few femtomoles (10^{-15} moles) of adduct. The disadvantage of ^{3}H in animal studies is that it is susceptible to loss during metabolism or by exchange reactions. These reactions can lead to misinterpretation of results. The use of ^{14}C will circumvent exchange and metabolism problems, but the detection limit is about 200 times higher than that achieved with ^{3}H (101). Because the general public is not exposed to radiolabelled carcinogens, this method would not be useful in epidemiological studies.

High performance liquid chromatography coupled with an ultraviolet absorbance detector has a limit of detection in the range of hundreds of picomoles (10^{-12} moles) of adduct. This method is useful for many adducts because many carcinogens show strong absorbance at 254 nm, the usual wavelength of the detector. Although this procedure could be used in the study of DNA adduct formation in humans, it is usually used to measure adducts obtained from experimental animals because the method is insensitive relative to other methods. Examples of DNA adducts that can be measured by using this technique are polynuclear aromatic hydrocarbons and benzidine derivatives.

Similarly, because polycyclic aromatic hydrocarbons are highly fluorescent molecules, fluorescence has been used to measure DNA adducts (219). The main disadvantage of this detection method is that it is relatively insensitive. Improvements in sensitivity were achieved by using a method called synchronous fluoresence spectrophotometry. This involves holding the difference between the excitation and emission wavelengths constant and scanning across the ultraviolet and visible spectrum. Detection limits as low as 31 femtomoles (10^{-15} moles) have been achieved with benzo(a)pyrene (219). Although this method is sensitive, is suffers from its lack of universality. It is only useful when studying compounds that fluoresce, and this property is primarily limited to polycyclic aromatic hydrocarbons.

Chemical derivatization coupled with gas chromatography/electron capture detection is also a viable detection technique. In this procedure, the DNA is broken down to nucleotides, and the nucleotides are derivatized to increase nucleotide volatility and to increase detector sensitivity. In a recent study, cytosine was derivatized with pentafluorbenzoyl chloride and dimethyl sulfate (66). As little as 50 nmol of starting material (cytosine standard) can be detected. Although this procedure shows some promise, it remains to be seen how well the derivatization procedure will work using 'real world' samples; the derivatization step may create too many interferences. It would be a convenient technique because of the wide availability of gas chromatographs.

Much literature has been published in the area of antibodies to carcinogen-DNA adducts. The determination of carcinogen-DNA adducts by immunologic procedures has certain advantages over other techniques. The sensitivity is frequently better than that obtainable with radiolabelled carcinogens. Antibodies are very selective for a particular three-dimensional structure and as such show very little or no cross reactivity with similar compounds. Immunologic assays are rapid, are highly reproducible, and can be used in situations where the cost of a radiolabelled compound would be prohibitive. Because one can measure nonradioactive DNA adducts, the procedure would lend itself to use in monitoring human tissues (174).

Antibodies to particular adducts are usually prepared by covalently binding the adduct of interest to a carrier protein or by using DNA that was modified _in vitro_. This is done because the size of a purine or pyrimidine base adduct is not large enough to stimulate an antigenic response. The protein-or DNA-adduct moiety is injected into an appropriate animal: rabbit, mouse, rat, etc. After a few months and several injections, the antibody titer is at maximal level (174). This is the general way in which polyclonal antibodies are prepared. As the name implies, the serum contains more than one clone of antibodies; there are thousands or even millions. Since each antibody has its own three-dimensional recognition site, the selectivity of polyclonal antibodies may or may not be exceptional.

If one could isolate only one antibody clone, one would expect to obtain maximal selectivity. This is the rationale behind the preparation of monoclonal antibodies. A preparation of monoclonal antibodies contains only one antibody clone. The antibodies are prepared by using hybridoma technology. Animals are innoculated with the antigen of choice in a manner identical to that used in polyclonal antibody production. The animals are sacrificed, and the spleen cells are removed. The spleen cells are fused with mutant myeloma cells under conditions such that only fused cells are viable. The fusion creates cells that can be cultured indefinitely. The antibodies with the desired specificity and sensitivity can then be located and selected. The spleen is a rich source of B cells, and it is B cells that produce antibodies. Therefore, each fusion that occurs between a B cell and a myeloma cell creates a cell that is immortal and produces a unique antibody. The main advantage of monoclonal antibodies is that they can be very selective. However, monoclonal antibodies do not necessarily have to be selective; for example, if a monoclonal antibody binds to a chemical site that is common to chemicals of a particular class (e.g., the hydroxyl portion of chlorinated phenols), selectivity would be poor. The main disadvantages of monoclonal antibodies are that they are expensive to produce, they are time-consuming to prepare, and luck plays a large part in locating an antibody with the desired specificity and sensitivity. Once the appropriate antibody is located, the assay is a very economical monitoring tool.

Polyclonal and monoclonal antibodies are used in various immunoassays: radioimmunoassay (RIA) including the radioimmunosorbent technique (RIST), enzyme-linked immunosorbent assays (ELISA), and ultrasensitive enzymatic radioimmunoassays (USERIA). All of these techniques could be used to monitor DNA adduct levels in human tissues.

RIA is a competitive assay where two identical haptens compete for the same antibody binding site. A hapten is a small molecule that can bind to an antibody but cannot by itself elicit an immunogenic response. One hapten, of known concentration and radiolabelled, is added to a fixed amount of antibody. The sample, containing an unknown quantity of hapten, is added, and the solution is allowed to attain equilibrium. The amount of radioactivity bound to the antibody will be inversely proportional to the amount of unknown hapten present. After equilibrium is reached, the antibody-hapten complex is precipitated. The amount of radioactivity is quantitated, and the level of unlabelled hapten is calculated by using a standard curve. The limit of detection in this assay is 5300 fmole of adducts or about one DNA adduct per 600 bases (benzo[a]pyrene; Table 2). Other examples of DNA adducts that have been quantitated by this method include several alkylated guanosines (153, 188, 233) and adducts from the carcinogen 2-acetylaminofluorene (176).

ELISA, as the name implies, uses an enzyme to measure the level of binding of a particular antibody to an antigen bound to a solid support (i.e., a microtiter plate). This is also a competitive assay. Essentially, an antibody is incubated in an antigen coated well in the presence of added antigen (DNA adduct sample).

The antibody is allowed to equilibrate between the free antigen in the solution and the antigen bound to the well. After equilibration, the well is washed, and a secondary antibody is added to the well. This antibody is specific for the primary antibody, and it binds to the bound antibody. The second antibody is conjugated to an enzyme that can react with an uncolored substrate to create a colored product. The free second antibody is washed out, and a substrate solution is added. The wells are incubated for a short period of time, and the enzymatically produced color is measured. The concentration of the added hapten can be calculated by using a standard curve. The limit of detection of this method in measuring benzo(a)pyrene-DNA adducts is 55 fmol or about one adduct in 5×10^{-4} bases (235). Alkylated guanosines (153) and aflatoxin-DNA adducts (72, 92) have also been measured by using this technique. The reason this procedure is so much more sensitive is that two amplification steps have been added. The first amplification arises from the fact that several secondary antibodies bind to each primary antibody, and the second amplification occurs because each enzyme molecule can convert many substrate molecules into colored products.

USERIA is a technique that is essentially identical to the ELISA technique except that the enzyme substrate is radiolabelled. The enzyme converts the radioactive substrate into a radioactive product. The product is chromatographically separated from the substrate, and the product is quantified. The detection limit for benzo(a)pyrene is about 10 fmole or about one adduct in 7×10^{-6} bases. USERIA has been used to quantitate several different types of adducts (72, 92, 97, 153).

RIST is very similar to ELISA and USERIA; the difference lies in the fact that the secondary antibody is radiolabelled. After the secondary antibody is bound to the primary antibody, the plates are washed, and the bound radioactivity is counted. The sensitivity of this procedure is about the same as the ELISA (153).

In summary, immunoassays possess many advantages; native, not digested, DNA is used for analyses, the procedure is quick and inexpensive, many samples

TABLE 3. COMPOUNDS TESTED FOR DNA BINDING *in vivo* BY ^{32}P-POSTLABELING ANALYSIS

		DNA adducts	
COMPOUND	TISSUE	No.[b]	Levels
Arylamines and derivatives			
2-Acetylaminofluorene	MS	6	++
	RL	11	+++
4-Acetylaminofluorene	RL	2	+
N-Hydroxy-2-acetylaminofluorene	RL	16	+++
N-Hydroxy-2-acetylaminophenanthrene	RL	10	+++
N-Hydroxy-4-acetylaminobiphenyl	RL	10	+++
N-Hydroxy-4-acetylamino-trans-stilbene	RL	9	+++
4-Aminobiphenyl	MS	1	+
Benzidine	MS	3	+
Azo compounds			
4-Dimethylaminoazobenzene	MS	2	+
Congo red	MS	2	+
Evan's blue	MS	1	+
Nitro compounds			
4-Nitroquinoline-l-oxide	MS	8	+
2,6-Dinitrotoluene	MS	3	–
Polycyclic aromatic hydrocarbons			
Benzo(a)pyrene	MS	5	+++
	RL	2	++
7,12-Dimethylbenz(a)anthracene	MS	8	+++
3-Methylcholanthrene	MS	13	+++
Benzo(e)pyrene	MS	5	+
Benz(a)anthracene	MS	2	+
Dibenz(a,c)anthracene	MS	6	+
Dibenz(a,h)anthracene	MS	3	++
Benzo(g,h,i)perylene	MS	2	++
Chrysene	MS	2	++
Anthracene	MS	ND[d]	–
Pyrene	MS/ML	ND[d]	–
Perylene	MS	ND[d]	–
Benzo(a)fluorene	MS	5	+
Benzo(b)fluorene	MS	5	+
Heterocyclic polycyclic compounds			
Dibenzo(c,g)carbazole	MS	7	+++
Dibenzo(a,i)carbazole	MS	6	+
Dibenzo(a,j)acridine	MS	2	++

(continued)

TABLE 3. (continued)

COMPOUND	TISSUE	DNA adducts	
		No.[b]	Levels
Alkenylbenzenes			
Safrole	ML	4	+++
Estragole	ML	4	+++
Methyleugenol	ML	4	+++
Myristicin	ML	3	+++
Dill apiol	ML	3	+++
Parsley apiol	ML	3	+++
Isosafrol	ML	2	++
Elemicin	ML	2	+++
Anethole	ML	2	++
Allylbenzene	Ml	2	++
Methylating agents			
N,N-Dimethylnitrosamine	ML	5	++++
1,2-Dimethylhydrazine	ML	5	++++
N-Methyl-N-nitrosourea	Ml	5	++++
Streptozotocin	ML	5	++++
Mycotoxins			
Aflatoxin B_1	RL	9[e]	++++
Sterigmatocystin	RL	15[e]	+++

[a] MS, mouse skin; ML, mouse liver; RL, rat liver. Adapted from (180).

[b] These numbers reflect the total number of adducts detected including those requiring very prolonged exposures for their detection.

[c] Total adduct levels: +, 1 adduct in > 10^7 nucleotides; ++, 1 adduct in 5 x 10^5 - 10^7 nucleotides; +++, 1 adduct in 10^4 - 5 x 10^5 nucleotides; ++++, 1 adduct in <10^4 nucleotides.

[d] Not detected.

[e] These adducts are oligonucleotides containing covalently bound carcinogen.

can be run per day and as such is a suitable method for screening large numbers of samples, and the method has been in use for several years. Immunoassays do have a few problems. The methods are not as sensitive as ^{32}P-postlabeling and may not be suitable for low exposure situations where sample size is limited. In addition, the antibodies must be characterized to determine what, if any, other adducts cross-react with a particular antibody.

Radiolabelled carcinogens allow for the detection of minute amounts of carcinogen binding to DNA but are not suitable for human studies. A method has been developed that has the sensitivity advantage of radiolabelled compounds, and it does not require that the carcinogen be radiolabelled. The method is referred to as ^{32}P-postlabeling, and the method is summarized as follows. Adducted DNA is isolated from a tissue source and is digested to form 3′-mononucleotides. ^{32}P is incorporated on the 5′-end of the nucleotides, and the adducts are separated by using multidimensional thin layer chromatography. This method has been used to screen over 70 compounds for their DNA adduct forming ability (76, 180, 181; Table 3). The separated adducts are quantified by using autoradiography. Because of the high specific activity of the ^{32}P, this method can detect adducts at about one adduct per 10^7 bases from a 1 ug sample of DNA (181). If the normal nucleotides can be removed before postlabeling, the detection limit can be lowered to about one adduct per 10^{10} nucleotides (74), to make this one of the most sensitive methods available. This level of sensitivity may be required when one is looking for DNA adducts induced by environmental carcinogens in the general population because of low exposure situations. Another advantage of this method is that one does not have to know the structure of the adduct in order to detect or quantitate it. In addition, a characteristic "fingerprint" is obtained for each adduct-forming compound. It is unclear how useful this "fingerprint" will be when adducts formed from several compounds are mixed together. Standards will be required to positively identify these presumably complex mixtures in order to establish a connection between adduct formation and exposure to a particular method. ^{32}P is a very strong beta emitter (approx. 1.7 MeV) and has a mean free path in air of about 20 feet. Stringent precautions must be taken in order to protect technicians and other laboratory personnel from radiation exposure. Also, the half life of ^{32}P is about 14 days so analyses must be carefully coordinated so as not to waste the ^{32}P.

LITERATURE REVIEW OF CARCINOGEN DNA ADDUCT STRUCTURE, PERSISTENCE, AND DOSE/RESPONSE CHARACTERISTICS

This section reviews DNA adduct research that has been published in the last 5 years. The review will be divided by chemical class and will be further subdivided by chemical. In addition, the information is summarized in tabular form in the appendix.

ARYLAMINES

An excellent review of the literature pertaining to the study of arylamine-DNA adducts has recently been published (203) and will be summarized here. Exposure to aromatic amines was first associated with human bladder cancer in dyestuff industrial workers, and much research has been conducted in the area of arylamine-induced genotoxicity.

1-NAPTHYLAMINE

Although 1-napthylamine (1-NA) is not carcinogenic, it can be chemically altered in vitro to form N-hydroxy-1-NA. N-hydroxy-1-NA is strongly carcinogenic at the local site of injection. When N-hydroxy-1-NA was reacted in vitro with DNA, two adducts were characterized: N-(deoxyguanosin-O^6-yl)-1-NA and 2-(deoxyguanosin-O^6-yl)-1-NA. When N-hydroxy-1-NA was injected into rats (16 umol), N-(deoxyguanosin-O^6-yl)-1-NA was the major adduct detected (22.6 adducts/10^6 nucleotides). The adduct was persistent; adduct levels dropped only 30 percent from one day after administration to seven days after administration (60). The reason 1-NA is not carcinogenic is probably because it is not metabolized to N-hydroxy-1-NA by the cytochrome P-450 monooxygenase system in vivo.

2-NAPTHYLAMINE

2-Napthylamine is a commonly used intermediate in the dyestuff industry. In contrast to its isomer, 1-NA, 2-napthylamime (2-NA) is carcinogenic in rats, dogs, and humans. Like 1-NA, its N-oxidized derivative N-hydroxy-2-NA formed derivatives with DNA when reacted in vitro (105). Three adducts were characterized; an imidazole ring-opened derivative of N-(deoxyguanosin-8-yl)-2-NA, 1-(deoxyguanosin-N^2-yl)-2-NA, and 1-(deoxyadenosin-N^6-yl)-2-NA. After administration of 2-NA to dogs (60 umol/kg), the three characterized DNA adducts were detected in both the liver (non-target organ; between 0 and 2 adducts/10^8 bases) and the bladder urothelium [i.e., the cells lining the bladder (target organ; between 0 and 10 adducts/10^8 bases)] (33, 106). The levels of the adducts were four times higher in the target versus the non-target organ. Seven days later, binding was measured in both organs. The N^2-deoxyguanosine adduct persisted in both the liver and urothelium whereas the C^8-deoxyguanosine adduct only persisted in the urothelium (33, 106). The persistence of the C^8-deoxyguanosine adduct in the bladder is therefore associated with the induction of bladder cancer and it may be that this adduct triggers a mutational event that is important in the initiation of bladder cancer.

4-ACETYLAMINOBIPHENYL (AABP) and 4-AMINOBIPHENYL (ABP)

4-Acetylaminobiphenyl (AABP) is a research chemical that is a mammary gland carcinogen in the rat. The presumed active metabolite of AABP is N-hydroxy-AABP. Attempts were made to characterize the adducts formed in liver and in mammary gland DNA (123b). Although acetylated and nonacetylated adducts were found, only the acetylated adducts were characterized. The adducts were identified as 3-(deoxyguanosin-N^2-yl)-AABP and N-(deoxyguanosin-8-yl)-AABP. In another study, the persistence of these adducts in rat liver was monitored by using a ^{32}P postlabeling technique (Dose-40 mg/kg). The adducts detected were N-(deoxyguanosin-8-yl)-AABP (1.5 fmol adduct/ug DNA), 3-(deoxyguanosin-N^2-yl)-AABP (2.4 fmol adduct/ug DNA), N-(deoxyguanosin-8-yl)-4-aminobiphenyl (ABP) (13.2 fmol adduct/ug DNA), and another unidentified nonacetylated adduct (75). The levels of these adducts were decreased 80, 62, and 77 percent 24 hours after an acute administration of N-hydroxy-acetylaminobiphenyl. However, after 29 days, 38 percent of the N^2 adduct still remained. Therefore, it appears that at least one of the adducts of AABP is very persistent.

4-Aminobiphenyl (ABP) is another research chemical that is a potent bladder carcinogen in dogs and that forms DNA adducts. When N-hydroxy-ABP, which is the active form of ABP, was reacted with DNA *in vitro*, three adducts were formed; N-(deoxyguanosin-8-yl)-ABP, N-(deoxyguanosin-N^2-yl)-ABP, and N-(deoxyadenosin-8-yl)-ABP (33). When dogs were treated with ABP, (60 umol/kg), the same three adducts were detected in dog bladder and liver DNA (adduct levels were between 0 and 800 adducts/10^8 nucleotides) (33). It is interesting to note that the levels of binding in both organs were high and essentially the same, but only the bladder is susceptible to this carcinogen. The persistence of total adduct levels was measured over 7 days. Adduct levels remained constant over the 7-day period. This is another instance demonstrating that DNA adduct levels alone are not an indicator of cancer risk, at least over the 7-day period measured.

4-ACETYLAMINO-4'-FLUOROBIPHENYL

If one adds a 4'-fluoro group to AABP, the compound becomes a renal, hepatic, and mammary gland carcinogen in rats. 4-Acetylamino-4'-fluorobiphenyl is used as a cancer research chemical. As one would predict by using the previous compounds as an example, N-hydroxy-4-acetylamino-4'-fluorobiphenyl (AAFBP) is the ultimate carcinogen (123a). In the rat both acetylated and nonacetylated adducts were detected, but only the acetylated adducts were characterized. The two acetylated adducts were identified as N-(deoxyguanosin-8-yl)-AAFBP. The persistence of the metabolites varied greatly (123a). The nonacetylated adducts were removed with a half-life of about 10 days; N-(deoxyguanosin-8-yl)-AAFBP was removed with a half-life of two days. 3-(deoxyguanosin-N^2-yl)-AAFB appeared to be a persistent lesion at least over the time frame measured.

3,2'-DIEMTHYL-4-AMINOBIPHENYL

3,2'-Dimethyl-4-aminobiphenyl (DMABP) is an arylamine research chemical that is a colon carcinogen in rats. The active metabolite is N-hydroxy-DMABP. With N-acetyl-N-hydroxy-DMABP (dose-0.5 mmol/kg), two adducts were found: N-(deoxyguanosin-8-yl)-DMABP and 5-(deoxyguanosin-N^2-yl)-DMABP (229). The adduct levels in the liver were about twice as high as those seen in the intestine (60 and 20 pmol adduct/mg DNA versus 40 and 10 pmol adduct/mg DNA, respectively). However, the basal rate of cell division was at least 20-times higher in the intestine than in the liver, so the adducts in the intestinal lining cells are much more likely to cause an error in replication. Because DMABP is a colon carcinogen but is not a liver carcinogen, the results indicate that the rate of cell division may be an important consideration is estimating carcinogenic risk.

2-ACETYLAMINOFLUORENE

2-Acetylaminofluorene (AAF) is a biochemical research chemical that is a carcinogen in a number of species and in a number of organs including the liver, mammary gland, intestine, and bladder. N-Hydroxy-AAF appears to be the ultimate carcinogen, and after a single administration of AAF or N-hydroxy-AAF to the rat (dose-15 mg/kg), the following adducts were detected in liver; N-(deoxyguanosin-8-yl)-AAF and 3-(deoxy-guanosin-N^2-yl)-AAF (123, 124). The first adduct had a half-life of 7 days whereas the second adduct was a persistent lesion in hepatic DNA. Later studies (141, 223) indicated the presence of one major nonacetylated adduct, N-(deoxyguanosin-8-yl)-2-aminofluorene (AF). This adduct was relatively persistent in rat liver DNA (31, 76, 141, 175). 2-AAF is one of the few compounds for which long term dosing studies have been performed. Sprague-Dawley rats were administered 2-AAF at biweekly intervals for up to 56 days (10 mg/kg; 31), and adduct levels were measured on each day of dosing and 14 days after each dosing. Male rats showed all three AAF adducts in hepatic DNA. N-(Deoxyguanosin-8-yl)-AAF was detected in male rats only and only on the day of dosing (1-1.8 pmol AAF/mg DNA). 3-(Deoxyguanosin-N^2-yl)-AAF was detected on days 1 and 14 and increased with additional administration of 2-AAF (0.2-3 pmol AAF bound/mg DNA). N-(Deoxyguanosin-8-yl)-AF was the major adduct observed in males (10-20 pmol AAF bound/mg DNA), and the levels remained approximately constant over 56 days. The female rats showed only the nonacetylated adduct, and it too was persistent (10-60 pmol AAF/mg DNA, from day 1 to day 56). Total adduct levels in the female were higher than in the male; however, female rats are resistant to 2-AAF-induced carcinogenesis. This again brings up the point that total DNA adduct levels alone are not directly correlated to cancer incidence. In another study (175, 176), rats were given either 0.02 or 0.04 percent AAF. Adduct levels increased with time until equilibrium (about 300 pmol adduct/mg DNA) was reached, equilibrium was reached in about three weeks. Both acetylated and nonacetylated adducts were detected initially although when equilibrium was reached, only nonacetylated adducts were present. Adduct formation was also detected in the kidney and mammary gland. In single administration and continuous dosing studies, only N-(deoxyguanosin-8-yl)-AF was detected in these tissues (6, 31, 175).

2-AAF also induces bladder and liver tumors in dogs (60 umol/kg). N-(Deoxyguanosin-8-yl)-AF was detected in both tissues (100 and 500 adducts/10^8 nucleotides, respectively); other minor adducts were detected in hepatic DNA (33). However, between days 2 and 7 after administration, 80 percent of the adducts were removed. Mice are susceptible to hepatic tumors when exposed to 2-AAF, and N-(deoxyguanosin-8-yl)-AF was the major adduct found in heptatic DNA (127).

A related compound, 7-fluoro-2-acetamidofluorene, has been studied in rats, and one adduct has been characterized [8-(N-fluorenylacetamidoguanine adduct] (189). However, this compound is a laboratory-created test compound with no real environmental significance.

BENZIDINE

Benzidine (BZ) is a compound that is the building block of an entire family of dyes known as benzidine dyes. Benzidine is a urinary bladder carcinogen in humans and dogs and a hepatocarcinogen in rats, mice, and hamsters. It is believed that benzidine is converted enzymatically to the carcinogenic molecules N-hydroxy-N'-acetyl-BZ and N,N'-diacetyl benzidine. The major *in vivo* adduct found in the rat (dose-111 umol/kg) was N-(deoxyguanosin-8-yl)-N'-acetyl-BZ (70 pmol adduct/mg DNA) with lesser amounts of N-(deoxyguanosin-8-yl)-N,N'-diacetyl-BZ present (5 pmol/mg DNA; 115, 138, 139). The persistence of the major adduct in the rat has been measured in two studies. One study showed a 60 percent decrease in binding from day 1 to day 7 after administration of N-acetyl-BZ (initial adduct level in rat liver-90 pmol BZ/mg DNA; dosed for one week in drinking water-80 ppm; 138). The second study found a 40 percent decrease in adduct binding between days 1 and 2 after administration with no further decrease in binding thereafter for at least 4 weeks (137a). Similar results are seen in the mouse. A single adduct was found in the mouse; N-(deoxyguanosin-8-yl)-N'-acetyl-BZ. Adduct levels were highest immediately after cessation of dosing, and although adduct levels dropped 50 percent after one day, adduct levels remained constant for at least another week (138). In hamsters, the above adduct was found in hepatic DNA, and its persistence was similar to that seen in the rat (115). After dosing at 111 umol/kg, the level of binding of the above adduct after one day in liver DNA was 33 pmol/mg DNA. After 7 days, the binding of BZ dropped about 60 percent. Benzidine forms bladder DNA adducts in dogs, but the adducts have not as yet been characterized. Most dyes that form covalent DNA adducts are metabolized to benzidine, and, therefore, benzidine adducts are detected. However, this is not always the case. Although the dye Direct Blue 6 does yield N-(deoxyguanosin-8-yl)-N'-acetyl benzidine, another larger adduct has been detected and characterized (116). The adduct was characterized as disodium 8-amino-2-[4-(N-deoxyguanosin-8-yl)-aminobiphenyl-4'-yl] azo-1-hydroxy-naphthalene-3,6-disulfonate. Therefore, if one is going to study azodye-DNA adducts, one might also want to measure the azo dye-DNA adducts instead of benzidine-DNA adducts as they would be indicative of the original dye from which the adduct was derived.

N,N'-DIMETHYL-4-AMINOAZOBENZENE, N-METHYL-4-AMINOAZOBENZENE, AND 4-AMINOAZOBENZENE

N,N'-Dimethyl-4-aminoazobenzene (DAB) is a dye that was one of the first arylamines studied in terms of adduct formation. DAB is more commonly known as Butter Yellow. Metabolic activation occurs by demethylation to form N-methyl-4-aminoazobenzene (MAB). MAB is then N-oxidized to form N-hydroxy-MAB. The ultimate carcinogen is believed to be N-sulfonyloxy-MAB. MAB has been used in most studies because it is more carcinogenic than 4-aminoazobenzene (AB). In the rat the major hepatic adduct found was N-(deoxyguanosin-8-yl)-MAB (135). However, this adduct is not persistent; 100 percent of the adduct is removed after 7 days (30, 32, 135). The minor adduct found, 3-(deoxyguanosin-N^2-yl)-MAB, was persistent; its level remained constant for at least 2 weeks (30, 32). If multiple doses of MAB were administered, a third adduct was found: 3-(deoxyadenosin-N^6-yl)-MAB (216). Rats were administered MAB at a level of 0.2 nmol/kg on days 1,3,5 and 8. The adduct levels increased from 0 to 2 adducts/10^6 nucleotides over the 8 days. In a long-term

(5 week) feeding study (217), all three adducts were found to increase with time. However, when dosing was suspended, only one of the three adducts, 3-(deoxyguanosin-8-yl)-MAB, was persistent. Similar results were found in the mouse; 3-(deoxyguanosin-8-yl)-MAB was a much more persistent lesion in hepatic DNA than N-(deoxyguanosin-8-yl)-MAB (213).

AB is carcinogenic in very young mice, and one adduct has been characterized in mouse hepatic DNA when the mice were administered 0.3 umol AB/g; N-(deoxyguanosin-8-yl)-AB at a level of 20 pmol adduct/mg DNA (54). When the same type of mice were administered DAB (0.3 umol/g), the above adduct was found (5.5 pmol/mg DNA) along with N-(deoxyguanosin-8-yl)-MAB (2.8 pmol/mg DNA) and 3-(deoxyguanosin-8-yl)-MAB (1.5 pmol/mg DNA).

2-ACETYLAMINOPHENANTHRENE

Although 2-acetylaminophenanthrene (AAP) is not carcinogenic, it binds to DNA both *in vitro* and *in vivo*. In a ^{32}P postlabeling study, it was found that the hepatic DNA adduct levels of AAP increased with time up to 24 h to 61 pmol/mg DNA and only decreased 50 percent after 29 days (75) after a single intraperitoneal injection of N-hydroxy-AAP (40 mg/kg). Many adducts were detected, but only one was characterized; N-(deoxyguanosin-8-yl)-aminophenanthrene. The same study showed that DNA adduct levels induced by 2-AAF, a potent carcinogen, were removed by about 95 percent 29 days after injection. The results suggest that even adduct persistence is not necessarily followed by tumor formation.

trans-ACETYLAMINOSTILBENE AND *trans*-4-DIMETHYLAMINOSTILBENE

trans-Acetylaminostilbene (AAS) and *trans*-4-dimethylaminostilbene (DAS) are research chemicals that are used as model arylamines. AAS and DAS are potent carcinogens in rats, particularly in the sebaceous glands of rats. A long-term (6-weeks) feeding study with rats showed that AAS bound to DNA, protein, and RNA in several organs in the rat, but there was no correlation between binding levels of the adducts and organ sensitivity to AAS (93). When organ exposure, as determined by protein adduct formation, was measured, there again was no correlation between exposure and organ sensitivity. It would have been interesting to measure DNA repair rates to see if any correlation existed between DNA repair and organ sensitivity.

trans-4-Dimethylaminostilbene (DAS) was employed to study the dose-dependence of adduct formation. The compound was administered to rats in doses ranging from 5 x 10^{-10} to 1 x 10^{-4} mol/kg. Binding of DAS to protein, DNA and ribosomal RNA was linear, and binding levels ranged from 0.5 to about 100,000 fmol/mg macromolecule (155). This report is important in that it provides experimental evidence that shows a carcinogen dose-response relationship can be linear over several orders of magnitude. It suggests that it should be possible to obtain usable dose-response relationships for carcinogens at the low levels to which people might be exposed.

POLYCYCLIC AROMATIC HYDROCARBONS

Polycyclic aromatic hydrocarbons (PAH) are widespread environmental pollutants that are produced primarily by industrial and transportational sources and can be found in materials such as soot, coal, tobacco smoke, and petroleum products. Because these compounds are carcinogenic in laboratory animals and because human exposure to these chemicals is widespread, much research has taken place in this area to determine if PAH's are carcinogenic in man. Epidemiologic studies have shown that industrial exposure to PAH's is associated with higher incidences of lung, skin, and bladder cancers (90, 112-114, 200). Cigarette smoking (16) and living in areas with high pollution (16, 89, 103, 172) are also associated with increased lung cancer incidence. All of the above conditions involve exposure to PAH's. The following is a summary of the research to date that characterizes the formation and persistence of PAH's. For a more detailed summary, an excellent review has been written by Stowers and Anderson (203).

BENZO(a)PYRENE

Benzo(a)pyrene (BaP) is the most extensively studied of the PAH's and is one of the most widespread in the environment. BaP is so widespread primarily because it is almost always associated with combustion. In our post-industrial revolutionary age, BaP is ubiquitous. It was one of the first carcinogens studied because it can be measured by using its UV absorption properties. BaP is a PAH that must be metabolized before it can form covalent adducts with DNA. After two epoxidation steps, the two major BaP metabolites that bind to DNA are (+)-7B,8a-dihydroxy-a,10a-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDEI) and (-)-7B,8a-dihydroxy-9B,10B-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene (BPDEII). These types of metabolites are known as diol epoxides, and most other PAH metabolites are of the same type. Studies have also been conducted by using human colon tissue to measure metabolism of BaP in humans. The two major metabolites found were those listed above (13).

BPDEI and BPDEII mainly bind to the 2-amino group of guanine residues, but they can also bind to the N-7 of guanine (159), adenine (108, 109, 140, 204), cytosine (204), and to phosphate residues (69, 121). BPDEI and BPDEII bind to lung, liver, colon, kidney, muscle, brain, and forestomach of the A/HeJ mouse and to lung, liver, colon, muscle, brain, and blood of the New Zealand White Rabbit (203). Other studies show adduct formation in lung, liver and kidney, colon, and intestine of mice (61). BaP also binds to DNA in the skin of several mouse strains (11, 18, 45, 52, 73, 122, 163, 168). The major adduct found in all cases was BPDEI bound to the N-2 position of guanine residues. Minor adducts are also seen bound to adenine residues. Once again, similar adduct formation is found in both susceptible and resistant organs. Adduct formation in the rat has also been measured; however, all of the adducts have not been characterized. One adduct has been identified in the rat (BPDEI-guanine). In rat lung, liver, and skin, it was a minor adduct (19, 39, 102) whereas in rat hepatocytes, it was the major adduct (10). Adducts have also been detected in humans (63,165)

Several dose-response studies have been conducted. In several mouse strains and in several organs, BaP levels in various organs have varied linearly (0.01-300 ug BaP/mouse, 2-11 umol/kg in the mouse; 3, 163) and non-linearly (11-135 umol/kg in the mouse, sigmoidal dose/respones;3). In both studies, the dose response curve was linear or approached linearity at the lower doses. If this linearity response at low doses is a general phenomenom for most carcinogens, it may be relatively easy to establish a dose-response relationship in a human pilot study.

In the rat, an increase of an intravenous administration of BaP from 1 umol to 10 umol increased adduct levels in rat lung approximately 5 fold and in rat liver about 3 fold (39). Although dose response linearity may not exist at high dose levels, it appears that the association of low levels of adducts with a given dose should be possible. One of the most interesting studies measured DNA adduct formation in cultured human, monkey, dog, hamster, and rat tissue [bladder and tracheobronchial tissues] (51). The patterns of metabolism were similar in all species; however, the level of adducts were widely variable. DNA adducts in human bladder were 30 times higher than in the rat. In the trachea-bronchus, the difference in adduct binding was about 10 fold between man and the rat.

BaP binding to proteins, both cellular (3) and circulating [i.e., hemoglobin (195)], has been measured. The dose-response curve was linear in both cases, and the increase in BaP-protein adducts correlated with the increase in BaP-DNA adducts. Therefore, measurement of BaP-protein adducts would be a good method for monitoring exposure to BaP in the general population. Studies have been run on human populations. One study measured BaP adduct formation in exposed and unexposed coke oven workers. Although the exposed group showed higher DNA adduct levels than the unexposed group, individual variation was great. Some exposed workers showed no measurable adduct formation (C.C. Harris, personal communication). This suggests that an estimate of individual DNA repair rates, enzyme levels, etc., may be necessary in order to interpret the DNA adduct level information.

The persistence of BaP-DNA adducts varies from species to species and from organ to organ; however, there is no correlation between adduct levels and the susceptiblity of a particular strain or organ to the carcinogenic effects of BaP (61, 125, 161, 168, 95). The following are examples of some of the studies that measured persistence of adducts. Pelling et al. (162a) measured the persistence of the (+) (active) and (-) (inactive) forms in mouse skin DNA. The adduct persistence for both compounds was about 2-3 days; the carcinogenic potential of these compounds differs by about 60 fold. Therefore, carcinogenic potential does not appear to be related to the persistence of BaP adducts in this tissue and mouse strain. Another study compared the persistence of several BaP adducts in mouse and rat skin epidermis (5). The main adduct, BPDEI-dG, had a half-life of less than a week in both species. However, 6 percent of the adduct remained in the mouse skin DNA after 3 weeks whereas the same adduct was completely removed after the same period of time in the rat. This shows that the simple measurement of a half-life may not give the entire story of persistence. Persistence very often tends to be biphasic with an initial rapid decrease in adducts which is followed by a slower adduct removal rate. Kulkarni and Anderson (125) measured the persistence of BPDEI-DNA adduct formation in the lung and liver of A/HeJ and C57BL/6J mouse strains. The half-life of the adducts in the A/HeJ mice were 20 and 14 days in the lung and liver whereas the half-life of the adducts in the C57BL/6J were 16 and 3 days, respectively.

The related compound 10-azabenzo(a)pyrene-4,5-oxide is a mutagen that can form DNA adducts (157) but that has no environmental significance. Another model compound that has been used to study PAH-induced adduct formation is 9-anthryloxirane. An in vitro study that reacted this compound with calf thymus DNA showed that the major adduct formed was through the N-3 position of adenine (237). Physicochemical studies have been conducted with the compound 1-oxiranylpyrene (117). This is a model compound that mimics the diol-epoxide of BaP but that does not contain any hydroxyl groups. This compound, although useful in kinetics experiments and other related experiments, has no environmental significance.

3-METHYLCHOLANTHRENE

3-Methylcholanthrene (3-MC) is a PAH that is commonly used in toxicological and pharmacological research as an inducer of the cytochrome P-450 monooxygenase system. It is also a carcinogen and is believed to be activated through a diol-epoxide in a manner similar to that seen with BaP (118, 119, 215). 3-MC has been shown to form DNA adducts in cultured human bronchus, colon, esophagus, pancreatic duct, and bladder (77). One study on the 3-MC-DNA adduct persistence in the mouse has been conducted. The persistence of adducts in four different mouse strains was measured. Adduct levels were measured at 4 hours, 7 days, and 28 days. In the lung, a susceptible organ designated as the A/J, C3H/HeJ, DBA/2J, and C57BL/6J mouse strains all showed persistence of DNA adducts from 19 to 62 percent after 28 days. In the liver, which is a resistant organ, all 3-MC adducts were removed after 28 days. This experiment suggests that there is a correlation between adduct persistence and the susceptibility to cancer. This is the exception rather than the rule concerning PAH adduct levels.

7,12-DIMETHYLBENZANTHRACENE

7,12-Dimethylbenzanthracene (DMBA) is one at the most powerful synthetic PAH carcinogens known. The binding and persistence of DMBA has been studied in mouse skin, rat liver, and cultured human tissue. DMBA forms DNA adducts in human bronchus, colon, esophagus, and pancreatic duct cultured cells (203); however, the adducts were not characterized nor quantified. Daniel and Joyce (50) injected Sprague-Dawley (susceptible) and Long-Evans (resistant) strains with DMBA (20 umol/rat). Five adducts were detected, but only two were partially characterized. The adducts were identified as DNA adducts with 1,2,3,4-tetrahydro-7,12-dimethylbenz(a)anthracene-3,4-diol-1,2-epoxide and with the diol-epoxide metabolite, 7-hydroxymethyl-12-ethylbenz(a)anthracene. Maximum levels of DMBA adducts were found in mammary gland DNA of the resistant and susceptible strain after 24 hr (20 and 18 umol DMBA/mol DNA, respectively). When persistence was measured up to 12 days after injection, the susceptible strain did not repair the adducts whereas limited adduct repair occurred in the resistant strain. Watabe et al. (226, 227) reported that the sulfate esters of hydroxymethyl-methyl-benz[a]anthracenes were mutagenic in the Ames assay. Dipple et al. (59) measured binding of DMBA to mouse skin DNA in two strains of mice, NIH Swiss and C57BL, after dermal application of 0.01 and 0.1 umol DMBA. Partial characterization of three adducts showed the formation of an anti-deoxyguanosine adduct and both a-syn and anti-deoxyadenosine adduct. The distribution of adducts was similar in both strains, with maximum adduct levels reached at 24 hours and with total adduct levels of 4.6 and 5.45 umol adduct/mol DNA (0.1 umol dermal application). At 48 hours, some decrease in adduct levels occurred. Total adduct levels dropped from 9 to 26 percent, the different levels were dependent on dose and strain. It should be noted that a ten-fold increase in the dermally-applied dose led to a 3- to 4-fold increase in the total DNA adduct levels.

15,16-DIHYDRO-11-METHYLCYCLOPENTA[a]PHENANTHRENE-7-ONE

15,16-dihydro-11-methylcyclopenta[a]phenanthrene-7-one (11-methyl ketone) is a PAH that is carcinogenic in mouse skin and lung, but not in mouse liver.

Several studies on the metabolism of 11-methyl ketone indicate that the major carcinogenic metabolite formed is a 3,4-dihydrodiol-1,2-epoxide (1, 47). The major DNA adduct appears to involve covalent bonding between the N2 of guanine and the C-1 carbon of 11-methyl ketone (231). One study has been conducted that determined the persistence of the DNA adducts produced by 11-methyl ketone in mouse skin, lung, and liver (2). Mice were injected with 3 mg of 11-methyl ketone, and the time couse of adduct removal was monitored for two weeks. Maximal adduct formation occurred about 2 days after injection. Total binding in the skin, liver, and lung was 283, 345, and 641 pmol adduct/mmol DNA. The half-life of the adducts in both skin and lung was about 6.5 days whereas the half-life of the adducts in the liver was about 2.5 days. When the DNA turnover rates were taken into account, it appeared that the lung and skin lesions were much more persistent than the lesions in the liver. It should be noted that maximal DNA adduct levels in the liver were twice as high as those seen in either the skin or lung; this suggests that rates of cell division are much more important than initial DNA adduct levels.

7-BROMOBENZANTHRACENE

One study has been located on this compound that measured the dose-response and persistence of 7-bromobenzanthracene (7-BMBA)-DNA adducts in mouse liver DNA, DNA associated proteins, and serum proteins (24). Mice were given an intravenous injection of 7.7 nmol 7-BMBA per gram body weight, and the time course of adduct formation was measured. Adduct formation reached a maximum in all targets measured in 0.5 to 3 hours, and adduct levels reached about 40 adducts/10^7 nucleotides in DNA. The dose-response curves for 7-BMBA was nonlinear for both proteins and DNA (24). Most of the 7-BMBA was rapidly removed from the mouse liver DNA within 1 day; however, a low level of 7-BMBA persisted for at least 1 month. 7-BMBA levels in albumin decreased rapidly with the loss of adduct corresponding to the normal turnover of the serum protein. Adduct levels decreased in the histone proteins, but it is not clear if the decrease represents protein turnover or adduct removal from the protein.

DIBENZ(a,h)ANTHRACENE

Dibenz(a,h)anthracene is a product resulting from incomplete combustion of organic materials. The only information located on DNA adducts formed by dibenz(a,h)anthracene is that adducts are indeed formed when cultured human bronchus, colon, and esophagus cells are incubated in the presence of this compound (77).

DIBENZO(a,e)FLUORANTHENE

Dibenzo(a,e)fluoranthene (DBF) is a PAH that binds to DNA. In an in vitro study, two diol-epoxide forms of DBF were reacted with calf thymus DNA (166). Acid hydrolysis and analysis of the nucleotides revealed that the active forms of DBF were reacted specifically with the exocyclic nitrogen of guanine (i.e., an N^2 guanine adduct). When mouse embryo fibroblasts were reacted with DBF, at least 6 adducts were formed (167). The persistence of 5 of the adducts was monitored for up to 48 hours. Some of the adducts were removed with an approximate half-life of 48 hours; however, others did not appear to be removed over the 48 hour period (167).

5-METHYLCHRYSENE

5-Methylchrysene (5-MC) is an environmental carcinogen that is found in tobacco smoke (158). This compound has two 'bay regions,' and, as one would expect, two active metabolites of 5-MC have been identified; both are diol-epoxides located at each bay region (144). Both compounds reacted with DNA in vitro, and the linkage to DNA was through the exocyclic nitrogen of guanine residues (144). These same adducts were detected in mouse skin DNA after dermal application of 5-MC (142). The two aforementioned adducts were detected in a ratio of 2.7:1 (3,4-epoxy versus 9,10-epoxy). Time course and persistence studies were conducted, and it was determined that the difference in adduct levels was attributable to differing levels of reactivity of the two diol-epoxides (143). The two adducts described were the major adducts detected, but other uncharacterized adducts were also present. 5-MC can be metabolized to 5-hydroxymethylchrysene (158). Okuda et al. (158) have reported that 5-hydroxymethylchrysene, when converted to a hydroxymethyl sulfate ester, is mutagenic in Salmonella typhimurium. This indicates that there are other metabolites in addition to the diol-epoxides that can form DNA adducts.

1-NITROPYRENE

1-Nitropyrene (1-NP) is a PAH that is found in diesel exhaust. Metabolic studies with 1-NP in Salmonella typhimurium and Chinese hamster ovary cells indicated that 1-NP undergoes nitroreduction to 1-aminopyrene (4, 96). The implication is that the reactive intermediate is N-hyroxy-1-aminopyrene. Several adducts have been detected in Salmonella, in Chinese hamster ovary cells, and in rabbit tracheal cells (87, 96, 98), and most have not been identified (28); but one adduct has been characterized in all of the tissues: N-(deoxyguanosin-8-yl)-1-aminopyrene. 1-NP is a mutagen, and a linear correlation was shown between the level of DNA adducts and the level of Salmonella revertants (96).

1,8-DINITROPYRENE

Another component of diesel exhaust that has been studied is 1,8-dinitropyrene (1-DNP). When Salmonella typhimurium was cultured in the presence of 1-DNP, the major adduct formed was N-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene (87). This implies that the reactive intermediate is 1-hydroxyamino-8-nitropyrene.

4-NITROQUINOLINE-1-OXIDE

4-Nitroquinoline-1-oxide (4-NQO) is a carcinogen that must be activated before DNA adduction will occur. Two compounds have been used as model ultimate carcinogens of 4-NQO: the O,O'-diacetyl and the O-acetyl derivatives of 4-hydroxyaminoquinoline 1-oxide (4-HAQO). These compounds, when reacted with DNA *in vitro*, formed five detectable adducts (three with guanosine and two with adenosine). One adduct was characterized; it was identified as N-(deoxyguanosin-8-yl)-4-aminoquinoline 1-oxide (21). When the relative rates of reactivity of the two model ultimate carcinogens were compared, the monoacetyl derivative was 2- to 3-fold more reactive with DNA (21). The reactivity of this more active derivative was studied using both native and denatured DNA. The levels of the one characterized adduct was 3.5 times higher in the denatured DNA (67). One would expect this, as active sites would be more accessible to attack in the denatured state. One problem with studies on this compound, as well as other compounds, is the fact that many adducts are not characterized. The one characterized adduct accounts for only 30 percent of the total number of adducts present after an *in vitro* incubation of the monoacetylated derivative of 4-NQO with denatured DNA (68). In a recent study, the levels of DNA adduct formation and the persistence of the adducts were measured in rat pancreas under conditions of high tumorigenicity (partially pancreatectomized) and low tumorigenicity (non-operated) (56). No difference was detected in adduct levels or persistence in the high and low tumorigenicity states. In other words, if the basal rate of cell division is increased, a given level of DNA adducts may be more likely to induce tumor formation. Another study measured unscheduled DNA synthesis (UDS), a measure of DNA damage, in mouse lung and liver DNA (126). 4-NQO is a carcinogen in mouse lung but not in liver. When UDS was measured in both tissues, DNA repair was measured in the lung but was not measured in the liver; this is a finding which is in apparent contradiction with the carcinogenic potential of 4-NQO. DNA adducts can also cause cell death as well as initiate tumor formation. When DNA was adducted *in vitro* with 4-HAQO, *E. coli* DNA polymerases could not replicate the entire length of the DNA; that is, arrest of DNA elongation occurred (238).

NATURAL PRODUCTS

There are many natural products, of a diverse nature, that are carcinogens. Such compounds as fungal and bacterial toxins and products produced by various plants are known to be carcinogenic. The literature on these compounds will be summarized in this section unless the compound is a simple alkylating agent, in which case it will be discussed in the section on alkylating agents.

AFLATOXIN B_1

Aflatoxin B_1 (AFB) is a toxin produced by the mold *Aspergillus flavus* that is highly toxic, mutagenic, teratogenic, and carcinogenic in a number of species (202). Aflatoxins have been reported to occur naturally in peanuts, peanut meal, cottonseed meal, corn, dried chili peppers, and other foodstuffs. Hepatic tumors are induced in several species after dietary intake of very low levels (parts per billion) of AFB. The FDA allows up to 15 ppb of AFB in foods because the incidence of liver cancer in the U.S. is so low. However, in countries where foods contain high levels of AFB, primary liver cancer is a relatively common occurrence.

AFB has a complicated metabolic pathway, but it is believed that the two metabolites that are responsible for AFB-DNA adduct formation are the 8,9- and 2,3- epoxide forms of AFB (8, 49, 163, 202). N^7-AFB adducts have been detected in several species, as has the imidazole ring-opened form of the above adduct (8, 43, 48, 49, 163, 202). The persistence of the N^7-AFB adduct is not great; it is less than 2 days, but the ring-opened adduct is very persistent; no loss of the adduct was measured over a 72 hour period after treatment of mouse embryo fibroblasts with AFB (8). In addition to studies that monitored the formation of DNA-adducts, the removal of DNA adducts has also been monitored. When rats were administered AFB, AFB-guanine adducts could be measured in the urine of the animals (35). The levels of adducts in the urine were nearly linear when compared with the dose (.125-1.0 mg AFB/kg), and the levels of DNA adducts in the liver correlated almost exactly with the levels in the urine.

STERIGMATOCYSTIN

Sterigmatocystin (ST) is another mold-produced toxin that is carcinogenic in a number of species (183). In a manner similar to aflatoxin B_1, ST is activated by metabolizing enzymes to a 1,2-epoxide form that can react with the N^7 of guanine (183). Although the ring-opened guanine structure has not been rigorously identified in vivo, it likely exists as it is chemically more stable than the non-ring-opened form (32, 183). The persistence of the adducts in rat liver appears to be triphasic. Half-lives of 12 hours, 7 days, and 109 days were measured (142). The persistence data may suggest that some adducts are more persistent than others or that some sites on DNA are more resistant to repair than others.

T-2 TOXIN

Although T-2 Toxin has been shown to bind to the DNA of cultured human esophagus cells (77), no reports of T-2 Toxin -DNA adducts have been located on this compound.

MITOMYCIN C

Mitomycin C (MMC) is an antibiotic agent that is also used as an antitumor agent. MMC forms both monoadducts and DNA crosslinks. After undergoing reduction, MMC can form covalent adducts. When activated MMC was incubated with DNA in vitro, three adducts were detected. Adducts were formed among MMC and the O^6 and N^2 atoms of guanine and the N^6 atom of adenine (84).

HYMENOXON

Hymenoxon (HYM) is a sesquiterpene lactone that is the primary toxic component in the plant commonly called bitterweed. An in vitro study was conducted to determine if HYM would bind to DNA. HYM formed adducts with guanosine, adenine, and cytosine, with guanosine adduct levels > adenine adduct levels > cytosine adduct levels (209).

FOOD PRODUCTS

There are many compounds in food that are carcinogens, either in their native form or after food preparation (i.e., cooking). Two reviews give an overview of this subject and will not be discussed here (7, 224). The following is a review of food products that form DNA adducts.

TRYPTOPHAN AND GLUTAMIC ACID PYROLYSIS PRODUCTS

When the amino acids tryptophan and glutamic acid are pyrolyzed during the cooking process, the products formed are 3-amino-1-methyl-5H-pyrido[4,3-b]-indole (Trp-P-2) and 2-amino-6-methyldipyrido-[1,2-a:3′,2′-d]imidazole, respectively (81-83, 85). These compounds are converted to the active form via the cytochrome P-450 monooxygenase system to the N-hydroxy derivatives N-OH-Trp-P-2 and N-OH-Glu-P-1 and have been shown to bind to DNA. The adducts have been identified as 3-(C^8-guanyl)-amino-1-methyl-5H-pyrido[4,3-b]indole (Gua-Trp-P-2) and 2-(C^8-guanyl)-amino-6-ethyl-dipyrido[1,2-a:3′,2′-d]imidazole (Gua-Glu-P-1) (82, 85). In addition to DNA adduct formation, these compounds also cause DNA strand scission through an oxidation-type process (225).

ALKENYLBENZENES

A number of alkenylbenzenes are found naturally in many essential oils that have been or are being used in food preparations or that are found in certain spices used in food preparations. The two most commonly studied compounds in this class are safrole and estragole. These compounds, as well as others of this compound class, are believed activated through the formation of a 1′-hydroxyl group. Further metabolism to expoxide, oxo, or sulfonoxy groups is believed to produce the ultimate carcinogens (170), and both safrole and estragole follow this metabolic pathway (169, 170, 234). Covalent adducts have been measured with safrole and estragole in rat liver DNA, transfer RNA, and cellular protein (169, 170). The DNA adducts have been identified as two N^2-deoxyguanosine adducts, a C^8-deoxyguanosine adduct, and an N^7-deoxyguanosine adduct (169, 170, 234). Using the ^{32}P-postlabeling technique, Randerath et al. (179) have measured the binding of the following alkenylbenzenes to rat liver DNA: safrole, estragole, methyleugenol, allylbenzene, anethole, myristicin, parsley apiol, dill apiol, elemicin, and isosafrole. The presence of some of the safrole adducts was detected at 140 days after administration of safrole (179), and some of the other alkenylbenzene adducts persisted for at least 43 days after compound administration (171).

PSORALENS

Psoralens are compounds of plant and synthetic origin that have been used extensively in clinical dermatology for treatment of diseases such as eczema and psoriasis. When the compounds are exposed to light at 360 nm, psoralens can form monoadducts with DNA, with the major adduct being a cycloadduct with thymine through the 5 and 6 positions (44). This psoralen-induced DNA damage inhibits or slows cell division and alleviates the symptoms of these conditions. Some psoralens can also form DNA crosslinks. Psoralens that have been studied include trimethylpsoralen, 8-methoxypsoralen, angelicin, and 5-methylisopsoralen. Further information on psoralens can be obtained in recent reviews of the subject (44, 101).

MONOCROTALINE

Monocroataline is an alkaloid present in the entire plant of *Crotalaria spectabilis*. Monocrotaline forms an adduct through the N^2 position of guanine (185).

CC-1065

CC-1065 is a naturally-occurring antibiotic found in *Streptomyces* that can form DNA adducts. CC-1065 forms an unusual adduct through the N^3 position of adenine, and it may function through inhibition of DNA synthesis (206).

THERAPEUTIC AGENTS

Many therapeutic agents form covalent adducts with DNA. The large majority of these agents are anticancer drugs. Most of the anticancer drugs are alkylating agents and will be discussed elsewhere.

CISPLATIN

One anticancer drug that is not strictly an alkylating agent, in the sense that it does not add an alkyl group to DNA, is *cis*-diamminedichloroplatinum(II) (cisplatin). Cisplatin destroys cancer cells by forming, among others, bidentate intrastrand N^7-deoxyguanosine adducts (177). Cisplatin is a direct-acting carcinogen; that is, it requires no metabolic activation. Cisplatin shows linear dose-response relationships in both man and experimental systems. Cisplatin adduct levels were measured in white blood cells of patients that had received 100-800 mg cisplatin/M^2 body surface area and adduct levels ranged from about 30 to 200 fmol adduct/mg DNA (177). When cultured Chinese hamster ovary cells were treated with 25 to 200 uM cisplatin, the level of DNA cross-links ranged from 0.3 to 2.5 interstrand DNA cross-links/10^9 daltons. The persistence of cisplatin in experimental animals is biphasic. The first half-life lies between 6 min and 1.5 hours whereas the second half-life was between 16 hours and 45 days. Because the dose received is known, a study of patients where correlations are made between the adduct levels and the enzyme, glutathione, etc. levels may be useful, both in determining whether the use of DNA adducts to estimate exposure is feasible and to improve the effectiveness of cisplatin in chemotherapy..

DIETHYLSTILBESTROL

Diethylstilbestrol (DES) is a synthetic estrogen that is a known carcinogen in humans and experimental animals. Although it is a carcinogen, little or no binding of DES to DNA has been determined, and, as a result, it was classified as a non-genotoxic carcinogen. Liehr et al. (133) conducted a long-term feeding study with DES in male Syrian hamsters where DES is a known renal carcinogen. Although no adducts were detected initially, three adducts were detected in the hamster kidney, a target organ, but not in the liver, a non-target organ. Because the measurement procedure does not give any information about the adduct structure, definite proof does not exist that a DES-DNA adduct was formed. The only certainty is that DNA adducts of some sort were formed.

ANTIPSYCHOTIC DRUGS

Two phenothiazine drugs, chlorpromazine and promethazine, have been shown to bind in *in vitro* and in bacterial systems to DNA and protein (55). The compounds were activated both through formation of a radical cation and through photoactivation with 350 nm light.

MISCELLANEOUS

The following compounds are a mixture of industrial chemicals and other compounds that do not fit into any of the above classes.

ACRYLONITRILE

Acrylonitrile is a compound that is produced in large quantity and to which about 300,000 workers are potentially exposed, particularly in the polymer industry. A recent study measured the *in vitro* binding of acrylonitrile to calf thymus DNA (201). The following adducts were detected by using mass spectrometry: 1-(2-carboxy-ethyl)-adenine, N^6-(2-carboxyethyl)-adenine, 3-(2-carboxy-ethyl)-cytosine, 7-(2-cyanoethyl)-guanosine, 7,9-bis-(2-cyanoethyl)-guanosine, imidazole ring-opened 7,9-bis-(2-cyanoethyl)-guanosine, and 3-(2-cyanoethyl)-thymine.

BENZYL CHLORIDE AND 4-CHLOROMETHYLBIPHENYL

Benzyl chloride is a chemical intermediate used in the manufacture of perfumes, pharmaceutical products, dyes, synthetic tannins, and artificial resins. No information was located on the sources of 4-chloromethylbiphenyl. The above compounds were found to damage the DNA of xeroderma pigmentosum-derived fibroblasts, but no information was located on the adducts formed (147).

STYRENE

Styrene is one of the most important monomers used in the plastics and synthetic rubber industry, and, as such, a fairly large number of workers are potentially exposed. Styrene requires metabolic activation to styrene 7,8-oxide before DNA adduction can occur. The major adducts formed are N^7-guanine adducts through the epoxide moiety (41). When rats were administered styrene or styrene 7,8-oxide, adducts were detected in liver, brain, lung, spleen, and testis DNA, and in hemoglobin and plasma proteins (41). When the dose-response was determined for liver DNA, hemoglobin, and plasma proteins, all were found to be non-linear.

HYDROXYLAMINE

In a report that described the mutagenicity of N^4-hydroxylcytidine, it was stated that N^4-hydroxycytidine was formed through the reaction of DNA with hydroxylamine (100).

DIMETHYLCARBAMYL CHLORIDE AND DIETHYLCARBAMYL CHLORIDE

Dimethylcarbamyl chloride (DMCC) and diethylcarbamyl chloride (DECC) are industrial intermediates that are use in the production of pharmaceuticals and carbamate pesticides. DMCC is a strong rat carcinogen whereas DECC is a weak carcinogen. When DMCC and DECC were reacted with calf thymus DNA *in vitro*, several adducts were formed; 6-dimethyl-carbamyloxy-2'-deoxyguanosine, 6-diethylcarbamyloxy-2'-deoxyguanosine, 4-dimethylamino-thymidine, and 6-dimethylamino-deoxyguanosine (191).

ALKYLATING AGENTS

There is a large class of compounds that either directly or through metabolic activation alkylate DNA. The vast majority of these compounds add methyl or ethyl groups to DNA purines and pyrimidines. Several excellent reviews have been written on this class of compounds (40, 58, 101, 148-150, 196, 197, 198), and they should be consulted for more detailed information. The literature summary will be subdivided into specific subclasses of alkylating agents.

ALKYL SULFATES

DIMETHYL SULFATE

Alkyl sulfates are industrial chemicals that are commonly used alkylating reagents. The two most commonly used alkylating agents are dimethyl sulfate and diethyl sulfate. When reacted with calf thymus DNA *in vitro*, dimethyl sulfate formed the following adducts: 3-methylguanine, 7-methylguanine, O^6-methylguanine, 1-methyladenine, 3-methyladenine, 7-methyladenine, and O^2-methylcytosine (156). The same adducts were detected (except for O^2-methylcytosine) when cell cultures were incubated with dimethyl sulfate (46,79,145). The levels of three adducts (7-methylguanine, 3-methyladenine, and O^6-methylguanine) in V79 cells treated with 8 and 15 ppm of dimethyl sulfate were measured. The levels of adducts induced at 15 ppm were 92.4, 12.0, and 0.5 umol/mol DNA (145). Only 7-methylguanine levels appeared to be directly related to the concentration of dimethyl sulfate. Also, the half-lives of two adducts were determined in the V79 cells. 7-Methylguanine and 3-methyladenine had half-lives of 14 and 4 hours, respectively (46).

DIETHYL SULFATE

Diethyl sulfate also forms adducts with DNA. When diethyl sulfate was reacted with DNA *in vitro*, the following adducts were formed: 1-, 3- and 7-ethyladenine, 3- and 7-ethylguanine, and 3-ethylcytosine (196).

ALKYL ALKANE SULFONATES

METHYLMETHANESULFONATE

Alkyl alkane sulfonates are research compounds used for alkylation reactions. The primary compounds that have been studied are methylmethanesulfonate (MMS) and ethylmethanesulfonate (EMS). MMS formed 3-methyladenine and 7-methylguanine adducts in *Chlamydomonas reinhardi* (207). The half-lives of the adducts were about 2 and 10 hours, respectively. The half-lives are very similar to those measured in V79 cells treated with dimethyl sulfate (46). When MMS was administered to female rats, both DNA and protein adducts were detected. After administration of 50 mg/kg of MMS, 12 ug of 7-methylguanine was excreted in the urine over 24 hours (64). The animals were sacrificed 7 days after dosing, and the hemoglobin level of S-methylcysteine attributable to the MMS was about 37 ng/mg hemoglobin.

ETHYLMETHANESULFONATE

When DNA was exposed to EMS, the following adducts were formed: 7-ethylguanine, 3-ethylcytosine, 3-ethyladenine and 1-ethyladenine (196). EMS also forms DNA adducts *in vivo*. When Wistar rats were administered 200 mg/kg EMS, 7-ethylguanine, 3-ethylguanine, 3-ethyladenine, and O^6-ethylguanine were detected in rat liver (57). The persistence of certain adducts were then measured. The half-life of 7-ethylguanine was about 6 days, and the half-lives for both 3-ethylguanine and 3-ethyladenine were less than 6 days. The exposure-dose relationship was explored in a study that exposed *Neurospora crassa* and *Saccharomyces cerevisiae* to EMS. The two strains were exposed to EMS concentrations from 2.5 to 50 mM. Ethylation (unspecified adducts) of DNA was linear but was less than proportional to the concentration of EMS (221). Ethylation ranged from 0.8 to 8.6 ethylations/10^4 nucleotides in *Neurospora crassa* and from 0.6 to 6.7 ethylations/10^4 nucleotides in *Saccharomyces cerevisiae*.

n-BUTYLMETHANESULFONATE

The alkyating potential of n-butylmethanesulfonate has been investigated. When reacted with DNA *in vitro*, the following adducts were formed: O6-n-butylguanosine, 7-n-butylguanosine, and 3-n-butylguanosine (187).

DIALKYL NITROSAMINES

N-NITROSODIMETHYLAMINE

Dialkylnitrosamines are a group of chemicals used widely in industry and in cancer research. N-Nitrosodimethylamine (DMN) is used in industry as an antioxidant, as an additive for lubricants, and as a softener of copolymers. N-Nitrosodiethylamine (DEN) has similar uses and is also used as a gasoline additive. Two animal studies were located on DMN. DMN was administered to mice, and adducts were detected via the ^{32}P-postlabeling assay. The adducts detected were 7-methylguanine and O^6-methylguanine (182). In another study, DMN (10 mg/kg) was administered to Wistar rats, and the following adducts were detected: 7-methylguanine (379 umol/mol DNA-P), 3-methylguanine (2 umol/mol

DNA-P), 7-methyladenine (<1 umol/mol DNA-P), 3-methyladenine (9 umol/mol DNA-P), and O^6-methylguanine (37 umol/mol DNA-P) (57). The half-lives of all of the adducts were less than 6 days.

N-NITROSODIETHYLAMINE

DEN was also administered (50 mg/kg) to Wistar rats. The adducts dectected were 7-ethylguanine (31 umol/mol DNA-P), 3-ethylguanine (3 umol/mol DNA-P), 3-ethyladenine (4 umol/mol DNA-P), and O^6-ethylguanine (2 umol/mol DNA-P) (57). Once again, the half-lives of all of the adducts were less than 6 days. Another experiment exposed rat hepatocytes to 40 ppm DEN, and the levels of O^6-ethylguanine and O^6-ethylthymine were measured at 8, 16, and 28 days. The levels of the ethylguanine decreased from 0.3 to less than 0.2 pmol adduct/umol guanine whereas the levels of ethylthymine increased from 5 to 10 pmol adduct/umol thymine (184). The authors postulated that the ethylated thymine adduct levels might be an important part of DEN-induced carcinogenicity.

MISCELLANEOUS NITROSAMINES

DNA adduct information was located on four other nitrosamines. N-Nitrosomethylbenzylamine (MBN) is a research chemical that was administered to Wistar rats. Four hours after an intravenous injection of MBN (0.017 mmol/kg), 7-methylguanine was detected in the esophagus, liver, lung, and forestomach, with the highest levels being in the esophagus (344 nmol/mol guanine) (94). N-Nitroso-N-acetoxymethyl-N-2-oxopropylamine (NAMOPA) is a model nitrosamine that is used to mimic the believed active form of N-nitrosodipropylamine in rats. When subjected to enzymatic hydrolysis in the presence of DNA, the following adducts were detected: O^6-methylguanine, 7-methylguanine, and 3-methyladenine (131). Another research chemical, nitrosobis-(2-hydroxypropyl)amine induces liver tumors in hamsters but not in rats. When this compound was administered to rats and hamsters (20 mg/animal), more adducts were found in hamster DNA (134). The levels of 7-methylguanine and O^6-methylguanine in hamster liver DNA were about 280 and 23 dpm versus about 40 and 0 dpm in rat liver DNA. Finally, the levels of O^6-methylguanine in the rat induced by the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) were measured by using polyclonal antibody techniques. F344 rats were injected 60 times (40 mg/kg) over the course of 20 weeks and nasal mucosa, lung, liver, kidney, esophagus, spleen, and heart DNA was analyzed for O^6-methylguanine. Measurable levels were found in the nasal mucosa (8 umol/mole guanine) and lung (11 umol/mole guanine) (42). If the rats were only given one injection (87 mg/kg), adduct levels were measurable in the nasal mucosa (219 umol/mol guanine), lung (13 umol/mol guanine), and liver (34 umol/mol guanine).

Many carcinogens have been associated with tobacco smoke, such as 5-methylchrysene, benzo(a)pyrene, and 13 N-nitrosamines. However, in snuff, N-nitrosamines are the only carcinogens found in significant amounts. The ability of two tobacco-specific N-nitrosamines [N'-Nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)] to alkylate DNA was studied. NNK, but not NNN, formed O^6-methyl-guanine when the compounds were administered to rats (42).

NNN forms a nornicotine-DNA adduct rather than a simple methyl group.

N-NITROSOUREAS

N-nitrosoureas are research chemicals and therapeutic agents that alkylate DNA. The vast body of DNA adduct research done with N-nitrosoureas has utilized the compounds N-methylnitrosourea and N-ethylnitrosourea.

N-METHYLNITROSOUREA

N-Methylnitrosourea (MNU) forms DNA adducts both in vitro and in vivo. When calf thymus DNA was incubated with MNU, O^6-, 3-, 7-methylguanine, 1-, 3-, 7-methyladenine, and O^2-methycytosine were detected (156). Some or all of these adducts have also been found in cells treated in culture (46,71,79,156), rats (65, 107), and mice (79).

Two limited dose-response studies were located. V79 cells were treated with 0.08, and 0.40 mM MNU and adduct levels of 7-methylguanine, O^6-methylguanine, and 3-methyladenine were measured 2 hours after incubation with MNU. The levels of 7-methylguanine (17.4 and 89.3 umol/mol DNA-P), O^6-methylguanine (2.1 and 11.7 umol/mol DNA-P), and 3-methyladenine (0.6 and 2.9 umol/mol DNA-P) were roughly proportional to the concentration of MNU present (46). However, another dose-response study with V79 cells did not show complete proportionality. The cells were treated with 30 or 60 ug/ml MNU, and the levels of 7-methylguanine, O^6-methylguanine, and 3-methyladenine were measured. 7-Methylguanine and O^6-methylguanine levels showed a proportional relationship with dose (50-102 and 7.5-14.4 umol/mol DNA-P, respectively), but 3-methyladenine levels did not (5.4-6.2 umol/mol DNA-P) (156).

Several studies have looked at the persistence of MNU-induced adducts. Although most adducts are removed rather quickly, some do appear to persist. Chinese hamster cells were incubated with MNU, and the adduct levels of 3-methyladenine, 7-methylguanine, and O^6-methylguanine were measured at 0 and 20 hours after incubation. The levels of 3-methyladenine and 7-methylguanine dropped 84 and 55 percent, respectively, but the levels of O^6-methylguanine adducts had not decreased at all (71). When female rats were treated with MNU, seven adducts were identified: 3-, 7-, O^6-methylguanine, 1-, 3-methyladenine, O^4-methylthymine, and an imidazole ring-opened 7-methylguanine (107). With the exception of O^6-methylguanine and the imidazole ring-opened 7-methylguanine, the half-lives of the adducts were all under 2 days. The half-life of O^6-methylguanine was about 3 days, and the half-life of the imidazole ring-opened adduct was greater than 21 days. The authors suggested that the ring-opened adduct might be important in the process of tumorigenesis because of its extreme persistence. Another study looked at the persistence of 7-methylguanine and O6-methylguanine and their relationship to susceptibility to MNU-induced tumorigenesis. Five strains of mice with widely varying susceptibilities to MNU-induced tumorigenesis were administered MNU. The absolute levels and half-lives of the two adducts were not appreciably different in any of the mouse strains (79). The author postulated that the levels of adducts and their persistence was not necessarily predictive of susceptibility to tumorigenesis. Another explaination might be that they were looking at the wrong adducts. Perhaps levels of the imidazole ring-opened 7-methylguanine adduct would have correlated with susceptibility.

N-ETHYLNITROSOUREA

N-Ethylnitrosourea reacts with DNA *in vitro* and *in vivo*. When ENU was incubated with calf thymus DNA, the following adducts were detected: 1-, 3-, 7-ethyladenine, 3-, 7-ethylguanine, 3-ethylcytosine, O^6-ethylguanine, O^2-ethyl cytosine, O^2-ethyluracil, and O^4-ethyluracil (196). O^2-ethylthymine, O^4-ethylthymine, O^2-cytosine, O^6-ethylguanine, 7-ethylguanine, and 3-ethyladenine were detected in rat brain, liver, spleen, intestine, muscle, kidney, lung, and also in human fibroblasts (196).

Two studies were located that explored the dose-response relationship of ENU-induced adduct formation. Rats were injected with ^{3}H-ENU (10 or 100 mg/kg) and the levels of O^6-ethylguanine, and 7-ethylguanine were measured 1 hour later in testis DNA. The levels of 7-ethylguanine increased as expected (1.79 and 17.3 umol adduct/mol guanine) whereas the levels of O^6-ethylguanine actually increased more than expected (1.31 and 18.1 umol adduct/mol guanine) (190). The authors attributed the greater than proportional adduct increase to the saturation of alkylation of selective protein sites at the higher ENU concentration. Another study measured the dose-response relationship of ENU *in vitro* and *in vivo* by using an immunoassay system. ENU reacted with DNA linearly over the concentration range of about 0.1 to 100 ppm yielding O^6-ethylguanine concentrations ranging from about 6 x 10^{-7} to 1 x 10^{-4} mol adduct/mol guanine (153). Also, treatment of rats with 3 to 100 mg/kg ENU resulted in a linear formation of O^6-ethylguanine from 0.3 to 30 umol adduct/mol guanine (153).

The half-lives of the adducts varied considerably. As was the case with MNU, 3-ethyladenine and 7-ethylguanine adducts were removed efficiently by Chinese hamster ovary cells, but O^6-ethylguanine adducts were not (71). In a study that measured the half-lives of O- and N- ethyl adducts in mammalian cells and in the rat, all of the half-lives were about the same; 30 to 60 hours (196). The only exception was 3-ethyladenine; its halflife was about 8 to 10 hours.

MISCELLANEOUS NITROSOUREAS

n-Propylnitrosourea has been reacted with calf thymus DNA, and interestingly, two types of adducts were found: not only the expected n-propyl adducts, 7-n-propylguanine and O^6-n-propylguanine, but also the *iso* derivatives, 7-*iso*-propylguanine and O^6-*iso*-propylguanine (151). During the reaction with calf thymus DNA, the propyl carbonium ion likely rearranges to a more stable electronic configuration that would lead to the formation of isopropyl adducts. n-Butylnitrosourea was reacted with calf thymus DNA, and both n- and *iso*-butyl adducts were formed (187).

The research chemical, N-(2-oxopropyl)-N-nitrosourea was reacted with calf thymus DNA, and the following adducts were detected: 7-, O^6-methylguanine, and 3-methyladenine (131).

Several compounds in this class are bifunctional alkylating agents; bis(chloroethyl)nitrosourea (BCNU), bis(fluoroethyl)nitrosourea (BFNU), and N-(2-chloroethyl)-N'-cyclohexyl-N-nitrosourea (CCNU). These compounds are used in the treatment of various cancers, and they appear to kill cancer cells by inducing intrastrand DNA cross-links (101).

NITROSOGUANIDINES

Reports were located on the alkylating ability of N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG). MNNG forms 7- and O^6-methylguanine adducts, and ENNG forms 3-ethyladenine, 3-, 7-ethylguanine, 3-ethylcytosine, 3-ethyluracil, O^6-ethylguanine, O^2-ethylcytosine, O^2-ethyluracil, and O^4-ethyluracil (26,196).

MISCELLANEOUS NITROSO COMPOUNDS

1-NITROSO-5,6-DIHYDROURACIL AND N-NITROSO-N-METHYLURETHANE

The anticancer agent and rat liver carcinogen, 1-nitroso-5,6-dihydrouracil is activated to form the electrophillic species 2-carboxyethylcarbonium ion. This species reacts with DNA to form the adduct 7-(2'-carboxyethyl)guanine (146). When rats and hamsters were given nitroso-2,6-dimethylmorpholine, two adducts were detected; 7- and O^6-methylguanine (134). The levels in the hamster (the susceptible species) were more than 10 times higher than those in the rat. Finally, N-nitroso-N-methylurethane was reported to form 7- and O^6-adducts *in vitro* (26).

HYDRAZINE DERIVATIVES

HYDRAZINE

Hydrazine derivatives occur both as synthetic and natural products including sources of food. Hydrazine, which does not contain any carbon atoms, induces DNA methylation. Both 7- and O^6-methylguanine adducts have been detected (101).

DIMETHYLHYDRAZINE

1,2-Dimethyl hydrazine (SDMH) is a potent colon carcinogen in rats and mice. Three adducts have been detected in these species; 7-, O^6-methylguanine, and O^4-methylthymine (27,91,99,184). Several studies have been conducted to determine if there is a relationship between DNA adduct levels and the carcinogenic activity of SDMH. SDMH was administered to two strains of rats, ICR/Ha (susceptible) and C57BL/Ha (resistant). Although a good correlation existed between adduct levels in particular segments of the colon and the incidence of cancer in the susceptible strain, no correlation was seen in the resistant strain (99). In another study, rats were injected weekly with SDMH, and the levels of 7- and O^6-methylguanine were monitored 1 week after each injection. No adducts were detected in the colon; only 7-methylguanine was detected in the liver, and both adducts were detected in the kidney (91). This does not correlate well with the fact that SDMH is primarily a colon carcinogen. The authors postulated that the rate of cell replication was an important factor in SDMH-induced carcinogenicity.

SDMH can induce liver tumors but only in hepatocytes and not in non-parenchymal cells (NPC). Rats were administered SDMH in drinking water (30 ppm), and 7- and O^6-methylguanine levels were monitored in hepatocytes and NPC's. Levels of 7-methylguanine were about the same in both cell types over the 28 day study, but levels of O^6-methylguanine were essentially 0 in hepatocytes and were found in NPC's at a concentration of 20 pmol/mg DNA (27). Similarly, administration of SDMH led to a increase in cell replication in NPC's but not in hepatocytes (132a). In addition O^4-methylthymine might be an important adduct in SDMH-induced carcinogenesis. When rats were administered SDMH, both O^6- and O^4- adducts were formed. Although the O^6- adduct levels were initially higher than the O^4- levels, the increased persistence of the O^4- adduct led to an increased relative level of the O^4- adduct over time (184). Many different methodologies were used in the above studies, including immunoassay (184) and ^{32}P-postlabeling methods (182).

HALOGENATED ALKYLATING AGENTS

The following list of chemicals are grouped together because of the presence of halogen atoms. Only one or two papers were located on each compound.

1,2-DIBROMOETHANE AND 1-BROMO,2-CHLOROETHANE

1,2-Dibromoethane is used as a soil, grain, and fruit fumigant, as an industrial solvent, and as a lead scavenger in gasoline. 1,2-Dibromoethane is a mutagen and is capable of producing liver, lung, stomach, mammary, adrenal, skin, spleen, and kidney tumors. 1,2-Dibromoethane was reacted with DNA *in vitro*, and it was determined that the rate of reaction with DNA was greatly increased in the presence of glutathione s-transferase (GSH s-transferase) (160). The same enhancement was seen when 1-bromo,2-chloroethane was incubated with DNA and GSH s-transferase. It is believed that the electrophillic species that reacts with DNA is the S-(2-bromoethyl)GSH episulfonium ion, and the adduct formed has been identified as S-[2-(7-guanyl)ethyl]GSH. What makes these compounds particularly interesting is the fact that GSH is involved with bioactivation of a carcinogen rather than with its usual role of detoxification.

EPICHLOROHYDRIN

Epichlorohydrin, 1-chloro-2,3-epoxypropane is a solvent for natural and synthetic resins, gums, cellulose esters and ethers, paines, varnishes, nail enamels and lacquers, and cement for Celluloid. Epichlorohydrin has been shown to form 7- and O^6-alkylguanines (26).

VINYL CHLORIDE

Vinyl chloride is an important industrial chemical that is used in the production of polyvinylchloride (PVC). Vinyl chloride is also a carcinogen. Its active form is chlorooxirane (i.e., ethylene oxide with an attached chlorine) (177a). It is both a mono- and bifunctional alkylating agent, attacking primarily adenine, cytosine, and guanine (177a, 196).

MISCELLANEOUS ALKYLATING AGENTS

The compounds described below are of a diverse nature, and only one or two literature citations were located on each one.

DICHLORVOS AND METRIFONATE

The organophosphorus pesticide, dichlorvos, was fed to rats, and the DNA from the lung, liver, heart, brain, kidney, testis, and spleen was isolated and pooled. The adduct 7-methylguanine was detected (236). Similarly, the pesticide metrifonate was administered intraperitoneally to rats. 7-Methylguanine was found in the liver and kidney. Maximal levels were acheived six hours after administration, and depending on the dose, the half-life of the adduct was 5 to 15 hours (53).

ETHIONINE

Ethionine is a carcinogen that was shown to bind to DNA, RNA, and proteins in the rat, but no information was given on specific adducts (130).

GLYCIDALDEHYDE

Glycidaldehyde, 2,3-epoxy-1-propanal, has been shown to alkylate guanine at positions 7 and O^6 (26).

GYROMITRIN

Gyromitrin (acetaldehyde N-methyl-N-formylhydrazone) is found in the false morel mushroom. When administered to rats by gavage, it was found that liver but not lung DNA contained 7-methylguanine (86).

1,3-PROPANE SULTONE AND BETA-PROPIOLACTONE

1,3-Propane sultone and beta-propiolactone are both strained cyclic compounds that can alkylate DNA. When 1,3-propane sultone was reacted with DNA *in vitro*, 1-, 7-, and O^6-alkylguanine derivatives were detected (88). In another *in vitro* study, beta-propiolactone formed alkylated 7- and O^6-alkylguanine adducts (26).

STREPTOZOTOCIN

Streptozotocin is an antibiotic used for the induction of diabetes in experimental animals and as a therapeutic agent in the treatment of pancreatic neoplasms. When Streptozo-toxin was administered to rats (21 mg/kg), the following adducts were formed: 3- and 7-methyladenine and 7- and O^6-methylguanine (34). The adducts were measured in the liver, kidney, intestine, brain, and pancreas. Adducts levels ranged from less than one pmol/umol guanine to 486 pmol/umol guanine.

RECOMMENDATIONS FOR FUTURE RESEARCH

In the previous sections of this report, discussions have been presented on methods for measuring carcinogen adducts, on biological fluids and tissues that can be used to monitor adduct formation, and on compounds that have been studied that form adducts. From the information presented, it is possible to propose candidate compounds for further study. It is also possible to propose detection methodology and biological samples suitable for large scale population monitoring. However, these tools alone are not sufficient for the interpretation of the data that would be obtained from such a study. Much more preliminary experimental data is required before such epidemiological data could be interpreted.

Figure 6 shows a diagram that traces the path of a chemical carcinogen from its presence in the environment to the onset of cancer. The main objective of monitoring human subpopulations for carcinogen-DNA adduct formation is to obtain a better estimate of the dose than is obtained from exposure data that is currently being obtained. A further objective of biomonitoring is to obtain better estimates of risk by monitoring specific damage caused by carcinogens. In reviewing Figure 6, it can be seen that both DNA adducts and protein adducts would provide a better estimate of dose and of risk because the adduct levels measured are obtained from the organism (man) in which one wishes to estimate dose. However, carcinogen-macromolecule adducts are not a panacea. Adduct levels, in most cases, cannot be plugged into a simple equation to receive the answer (dose levels in man). Many steps in the process from compound uptake to the onset of cancer are either poorly understood or extremely complicated.

The dose response of many carcinogens has been studied in experimental animals, and almost all show at least some linearity in the dose response curve. However, much research still remains to be done. Most studies have used concentration ranges of only one or two orders of magnitude, and the dosing levels were very high (to facilitate adduct detection). Very little is known about dose response at very low levels of carcinogens. Any compound that is chosen for a human study should have animal experimental data that shows the dose response at very low levels since this is the level at which most exposures are likely to occur. Another problem with most present animal studies is the method of dosing. Most studies dosed animals by administering a single dose of the carcinogen being studied. In a real life situation, dosing would very likely be semi-continuous and perhaps intermittent. Additional animal research needs to be conducted so there will be a better understanding of dose-response under these dosing conditions.

The second step in the process from exposure to onset of disease is metabolism and distribution in the body. Metabolism is not as much of an issue with direct-acting carcinogens because the compounds form adducts without requiring metabolic activation. However, metabolism can still inactivate direct acting carcinogens. The manner in which the compounds are distributed throughout the body is important in determining what tissues are exposed to adduct-forming compounds. Animal studies that have measured the toxicokinetics of these compounds can be used to estimate how compounds are distributed in the body, so it should be possible to make reasonably accurate estimates of the dose received by an individual if protein adducts are quantified. The estimation of dose received of an indirect-acting carcinogen is not as easy. In order to make a reasonable estimation of the dose received over the period of exposure measured by protein adducts, one needs information on the level of metabolic activity in the individual from which the samples are taken. The measurement of enzyme levels will be difficult, as the highest concentration of most xenobiotic-metabolizing enzymes is found in the liver. Liver biopsies are out of the question in most cases, so estimations of enzyme activity will have to be made via other means. Perhaps the measurement of the levels of endogenous metabolites will make it possible to estimate enzyme levels.

The use of carcinogen-DNA adducts as a measure of dose received is likely to be less accurate than the use of protein adducts. The main reason for the decreased accuracy of DNA adducts as a measure of exposure is that the adduct levels are altered via DNA repair and dilution through cell division. Protein adducts also decrease with time, but the decrease can be accurately estimated because the half-lives of the proteins are accurately known. There is much less known about DNA repair, and hence, the uncertainty associated with dose would be greater.

DNA adducts would be much better to use as an estimate of cancer risk because one is measuring the actual damage to the cellular genetic information. However, the levels of DNA adducts would at best provide a rough estimate of risk for the following reasons. Prior damage to DNA might not be measurable as DNA adducts because the adducts may have already induced a mutation. The mutation would not be measurable as an adduct, so past DNA damage could be hidden. Also, not all adducts are associated with increased incidence of cancer. Research must be conducted to determine what adducts are associated with increased cancer risk. Finally, very little is known about how mutations trigger the onset of cancer. Optomistically, it will be several years before the progression from mutation to cancer onset.

The preceding has pointed out some of the inadequacies in present knowledge of the relationship between dose received, carcinogen-macromolecular adduct formation, and the onset of disease. Although much research still needs to be conducted before the measurement of adducts would be useful, the potential for their use in exposure assessment is great. The measurement of carcinogen-macromolecule levels in an organism for dose estimation is inherently more accurate than the measurement of carcinogen levels in the environment.

RECOMMENDATIONS FOR TISSUES AND FLUIDS TO BE ANALYZED

There are two main points to be considered when choosing fluids or tissues to detect adduct formation. The first point is one of invasiveness. If the sample collection technique is too invasive, it will be impossible to obtain enough volunteers for the studies. The second point involves the usefulness of the sample. The further away the sample is from the target organ, the less representative the measured adduct formation will be of the adduct levels at the target site unless animal studies indicate otherwise. Therefore, tissue and fluid selection will be a compromise between these two points.

The best compromise fluid is blood. Sample collection is relatively non-invasive, and it contains several molecules that can contain covalent adducts.

White blood cells contain DNA; therefore, DNA adduct formation can be monitored. However, one must remember that the DNA adduct level may not correlate with the DNA adduct level in the target organ(s) or with the dose received. In addition, protein adducts can be monitored. The two main proteins to be monitored would be hemoglobin and human serum albumin. Each protein would give different levels of integration data as the half-lives of the proteins are 120 and 20 days, respectively. Also, human serum albumin is extra-cellular and is not protected by a cellular membrane; it might show a higher level of protein adduct formation. In summary, both integration and equilibration data can be obtained from blood.

Urine is also an excellent choice for monitoring DNA adducts. When DNA repair occurs, the removed adducts are excreted in the urine. However, not many carcinogen adducts have been monitored in the urine, so additional animal studies would have to be conducted. In one animal study, the levels of aflatoxin-DNA adducts found in the urine correlated with the administered dose of the carcinogen. Theoretically, any adduct that is repaired could be monitored by using this fluid.

Tissues obtained during a biopsy or autopsy would provide the most accurate information concerning the level of damage that occurs at the target site. The problem with biopsy samples is that they are very difficult to obtain, and it would be difficult to obtain volunteers for such a study. The question that needs to be asked is if target DNA adduct level information is really needed. If the main purpose of the planned studies is to obtain dose information, that is, levels of the carcinogen that have entered the body, then the information obtained from adducts measurable in blood may be sufficient.

In summary, the best approach to monitoring human subpopulations would entail collection of both blood and urine samples. This approach would allow the collection of the most data with a minimum of discomfort to the individuals.

SUGGESTED MEASUREMENT TECHNIQUES

Biomonitoring implies the collection of several biological samples over a period of time from many individuals. As a result, many samples will have to be analyzed. Also, exposures to the carcinogen(s) of interest will normally be at extremely low levels, so the adduct levels will likely be at or below the detection limit of most methods. Because the adduct levels will be very low, interferences may present a serious problem. The above facts require that the methods be inexpensive, fast, very sensitive, and selective. Of all the adduct detection methods available, only two appear to lie within the above constraints: immunoassay techniques and ^{32}P-postlabeling techniques. The immunoassay technique, except for the initial antibody identification, isolation, and preparation, is the simpler of the two techniques. The ^{32}P-postlabeling technique, although more cumbersome, can be more sensitive. A possible method to monitor for DNA adducts would include a rapid screening with the immunoassay method followed by the ^{32}P-postlabeling method for those that showed up negative on the immunoassay screening. This procedure would be the most cost effective as only those samples with very low levels of DNA adducts would be analyzed by the more expensive ^{32}P-postlabeling method.

SUGGESTED COMPOUNDS FOR BIOLOGICAL MONITORING

Biological screening of large populations to determine the presence of DNA adducts will likely prove very expensive, no matter how inexpensive assays are. The process of tracking large numbers of people is very expensive in and of itself. Therefore, compounds selected for monitoring should be those for which there is a high expectation of demonstrating a useful exposure-dose relationship cost effectively. An economic criteria that should also be included is the presence of relevant preliminary research. A lot of preliminary animal data will be needed before a pilot human study can be conducted. If a large part of data needed has already been published on particular compounds, serious consideration should be given choosing one of them. However, there are probably few, if any, compounds that have a large part of the preliminary research already completed.

An important criteria to be considered in the selection of a compound for a pilot study is the existence of exposed populations. Coupled with this would also be the criteria that an unexposed population also exists. For example, studies have been conducted where populations were monitored for the presence of benzo(a)pyrene adducts. The studies showed that benzo(a)pyrene was so ubiquitous in the environment that adduct levels above background were only detected in persons exposed to high levels of the compound. In other words, the background levels of benzo(a)pyrene-DNA adducts were so high that they masked any dose-response curve that was attributable to occupational exposure.

The EPA has a list of priority compounds that should be considered for further study with this technique of exposure assessment. It would benefit the Agency if one of the priority compounds were chosen. However, the presence or absence of a compound on the list should not be the overriding consideration. The primary consideration should be a compound that has the highest expectation of producing a usable exposure-dose relationship. Therefore, human population studies in which information on exposure is already quantitatively known might prove useful to determine the feasibility of using carcinogen-macromolecule adducts to monitor exposure to genotoxic chemicals. An ideal population for an initial study would be patients receiving anticancer drugs.

For example, the Health Effects Research Laboratory (HERL) in the Research Triangle Park is conducting an exposure-dose relationship study with 2,5-diazeridinyl-3,6-bicarboethoxyamino-1,4-benzoquinone (AZQ). AZQ is a chemotherapeutic agent for brain tumors. Unfortunately, long term studies are difficult because of the extremely poor prognosis for patients with brain tumors.

Another chemotherapeutic agent, cisplatin, would be ideal to research the exposure-dose relationship. Current research indicates that this therapy is effective in about 50 percent of patients receiving this treatment. In those patients where treatment is effective, DNA adducts are detected in circulating white blood cells, whereas adducts were not detected in patients resistant to cisplatin treatment. This suggests that there are factors that influence the levels of cisplatin in the vicinity of DNA, such as drug metabolizing enzyme levels, glutathione levels, or permeability of the membrane to cisplatin. This population would provide an ideal setting to determine if a biomarkers package can be developed to correct for individual variation in the above factors. This study might involve the monitoring of (1) DNA adducts in white blood cells or the target organ (biopsy), (2) DNA adduct levels in urine to estimate DNA repair, (3) Thioether compounds in urine to monitor glutathione levels, (4) glutathione levels in the blood, and (5) cisplatin-protein (Hb and serum albumin) adducts in the blood. Also, in vitro studies of cancer cells in culture might also be used to estimate rates of DNA repair or to measure cell membrane permeability to cisplatin. This list of variables to monitor is by no means complete and is given only to suggest some variables that may be important. This study to look at a biomarkers package with patients receiving cisplatin will not only test the potential of using a biomarker package to measure exposure but should also lead to a more effective chemotherapeutic treatment. It is proposed that a study of this nature be used to support the Office of Research and Development (ORD) rotational assignment or sabatical programs under the aegis of the EPA in order to improve the scientific quality of personnel, and to give personnel the opportunity to participate in research and to contribute to publications. Such an agreement could be established with NCI, Bethesda, MD, where research on DNA adduct measurements in relation to cisplatin is currently being conducted. NCI has expressed interest in conducting a study of this nature if the appropriate manpower can be supplied.

The analysis of alkylated DNA and protein adducts may be a useful monitoring project. Alkylating agents are widely distributed compounds, and they have been detected in some foods or food byproducts as well as in tobacco products. In the past, there has been some debate as to whether these compounds are a threat to the general population because of their short half-life in the body. It is this short half-life that also makes the measurement of the free compound in tissues or fluids impossible (42). Therefore, measurement of adducts may provide a method for estimating the dose of alkylating agents received by individuals. A population of exposed individuals is readily available: persons who use tobacco products. Estimating exposure to specific alkylating agents present in tobacco products should be fairly simple because the concentration of alkylating agents in the tobacco and the amount of tobacco product used can be measured easily. In addition, the dosing regimen is relatively consistent, as tobacco usage would occur on a more or less regular basis. Smokeless tobacco may be a good choice because levels of the alkyating agents 4-(methylnitrosoamino)1-1-(3-pyridyl)-1-butanone (NNK), and N-nitrosonornicotine (NNN) are very high and because pyrolysis-produced carcinogens such as benzo(a)pyrene, 5-methylchrysene, and dibenz(a,h) anthracene are not present. An antibody exists for O^6-methylguanine, and it has been used in at least one study to measure DNA damage induced by activated NNK (42). The use of this antibody may be complicated because it showed cross-reactivity with other methylated bases and some cross reactivity with native guanine. Although the cross-reactivity with native guanine is low, it would be an interference if the levels of the methylated guanine that are being measured are low. This problem can be overcome by using high performance liquid chromatography to remove the unmodified guanine residues from the sample. Of course, this will add to the cost of each assay. Although more difficult, methylated bases can be monitored by using the ^{32}P-postlabeling technique, and low detection limits should be possible. NNN would also be a good tobacco-derived alkylating agent to measure because the NNN-DNA adduct can be easily attributed to tobacco. NNK-derived DNA adducts are the same as any other methylating agent. Therefore, methylated DNA cannot be attributed to any specific methylating agent.

One compound that may be a good choice is the mycotoxin aflatoxin-B_1. Various levels of aflatoxin exposure exist. Corn and peanut farmers and handlers would likely be more highly exposed than others, and the general population would be exposed through peanut and corn food products. A problem that might exist would be the lack of negative controls. If all individuals have a high background level of aflatoxin B_1 adducts, then a reasonable dose-response relationship may not be found. It would be very useful if an exposure-dose relationship can be demonstrated because analytical methodologies for measuring levels of aflatoxin in the environment are either inaccurate or very complex. Excretion of aflatoxin-DNA adducts has been detected in laboratory animals that were administered aflatoxin, and a useful exposure-dose relationship was established. Also, the large, hydrophobic adduct produced by aflatoxin is amenable to analysis by ^{32}P-postlabeling methods so that low levels of adducts could be detected.

Other compounds have been located that did not have many references on adducts in the computerized computer search but probably should be given consideration for further study. Vinyl chloride is an industrial chemical that about three million workers are exposed to. Its primary use is in the manufacture of polyvinyl chloride (PVC). Vinyl chloride binds to hemoglobin when administered to rats (64); this suggests that vinyl chloride may also form adducts with DNA. Other chemicals in the polymer industry might also be good future candidates for study such as acrylonitrile, epichlorohydrin, and styrene. Styrene (in the form of styrene oxide) has been shown to form DNA adducts, and the pertinent literature has already been summarized. The industrial intermediate dimethylcarbamyl chloride, used in the production of pharmaceuticals and carbamate pesticides, might also be of interest. One might also look at methylated adducts formed by the pesticides dichlorvos and metrifonate. However, both pesticides methylate DNA, so the adducts would not be directly traceable back to the pesticides. o-Toluidine hydrochloride is another industrial chemical that is used in the manufacture of various dyes. The compound is a carcinogen, and a large number of workers in the dye industry are exposed. Other arylamines in the dye industry may also prove useful, such as benzidine or 2-napthylamine. Since benzidine is a metabolite of most benzidine-type dyes, benzidine-DNA adducts may indicate exposure to benzidine-type dyes. Exposure to the arylamine 4-aminobiphenyl has been measured (199, 212), so this comound should also be given consideration. Finally, psoralens might also prove useful as test compounds. These compounds are used therapeutically for a variety of skin diseases. If adducts are found in the blood or urine after treatment, it may be that an exposure-dose correlation can be found. At present, the EPA is conducting a exposure-dose feasibility study with the compound Psoraben in a collaborative study with Dr. Regina Santelli of Columbus University. They are looking at the dose-response of adducts formed in hemoglobin and WBC DNA.

In addition, several compounds have been identified that are on the EPA priority list and that may form adducts. Although no literature has been located on these compounds, it may be useful to carry out some preliminary experiments to determine if these compounds form adducts.
The compounds are:

1) Dichloromethane
2) Carbon tetrachloride

3) Polychlorinated biphenyls
4) Chloroform
5) Toluene
6) Formaldehyde

An initial study with these compounds might include a simple feeding study to determine if the compounds do form adducts. If so, several log dose-response experiments should be conducted to check linearity with Hb, serum albumin, and DNA.

This is not meant to be an all inclusive list as much as it is meant to comprise suggestions for compounds to be considered for environmental monitoring. Both immunoassay and ^{32}P-postlabeling techniques should be adaptable to nearly any DNA adduct that is characterized. Protein adducts can only be monitored by using immunoassay techniques because the ^{32}P-postlabeling technique is specific for DNA adducts. However, protein adducts should occur at higher concentrations than the DNA adducts because of a lack of protein adduct repair systems and because greater amounts of sample are available. Therefore, sensitivity should not be an insurmountable problem.

APPENDIX

SUMMARY OF DNA ADDUCT LITERATURE

The appendix summarizes the characteristics of DNA adducts that have been published in the literature. The information is derived primarily from literature from 1981 to the present, although some earlier literature is included.

The table is organized as follows. The compounds are in the order discussed in the text with simple alkylating agents listed last, again in alphabetical order.

The Appendix is self explanatory except for the DNA adduct persistence data. The data is presented in two ways: either as half-life data, or as percent increase (%I) or percent decrease (%D) over a time period (H-hour, D-day).

Other abbreviations are as follows:

-- - Information not given in literature
N/A - Not applicable

If a section is left blank, it indicates that the literature was not available and that that particular information was not given in the citation abstract.

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
1-Napthylamine (1-NA)	N-Hydroxy-1-NA	N-(deoxyguanosin-O^6-yl)-1-NA	Radioisotope
1-Napthylamine (1-NA)	N-Hydroxy-1-NA	2-(deoxyguanosin-O^6-yl)-1-NA	Radioisotope
1-Napthylamine (1-NA)	N-Hydroxy-1-NA	N-(deoxyguanosin-O^6-yl)-1-NA	Radioisotope
1-Napthylamine (1-NA)	N-Hydroxy-1-NA	2-(deoxyguanosin-O^6-yl)-1-NA and N-(deoxyguanosin-O^6-yl)-1-NA	MS
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	Imidazole ring-opened derivative of N-(deoxyguanosin-8-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	1-(deoxyguanosin-N^2-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	1-(deoxyadenosin-N^6-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	Imidazole ring-opened derivative of N-(deoxyguanosin-8-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	Imidazole ring-opened derivative of N-(deoxyguanosin-8-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	1-(deoxyguanosin-N^2-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	1-(deoxyguanosin-N^2-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	1-(deoxyadenosin-N^6-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	1-(deoxyadenosin-N^6-yl)-2-NA	Radioisotope
2-Napthylamine (2-NA)	N-Hydroxy-2-NA	1-(deoxyguanosin-N^2-yl)-2-NA, 1-(deoxyadenosin-N^6-yl)-2-NA and a ring-opened deoxyguanosine adduct	MS

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
N/A	N/A	In vitro	N/A	104
N/A	N/A	In vitro	N/A	104
N/A	30% D D1--D7	Rats	Inj. Site	60
--	N/A	In vitro	--	104
N/A	30% D D1---D7	In vitro	N/A	105
N/A	30% D D1---D7	In vitro	N/A	105
N/A	30% D D1---D7	In vitro	N/A	105
60μmol/kg(A); ~2 adducts/10^8 bases	100% D D2--D7	Dog	Liver	33
60μmol/kg(A); ~10 adducts/10^8 bases	10% D D2--D7	Dog	Bladder	33
60μmol/kg(A);$0<X<3$ adducts/10^8 bases	50% D (est.) D2--D7	Dog	Liver	33
60μmol/kg(A);$0<X<10$ adducts/10^8 bases	30% D (est.) D2--D7	Dog	Bladder	33
60μmol/kg(A);$0<X<3$ adducts/10^8 bases	50% D (est.) D2--D7	Dog	Liver	33
60μmol/kg(A);$0<X<10$ adducts/10^8 bases	30% D (est.) D2--D7	Dog	Bladder	33
--	N/A	In vitro	N/A	105

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
4-Acetylaminobiphenyl (4-AABP)	N-Hydroxy-AABP	3-(deoxyguanosin-N^2-yl)-AABP	Radioisotope
4-Acetylaminobiphenyl (4-AABP)	N-Hydroxy-AABP	N-(deoxyguanosin-8-yl)-AABP	Radioisotope
4-Acetylaminobiphenyl (4-AABP)	N-Hydroxy-AABP	N-(deoxyguanosin-8-yl)-4-aminobiphenyl	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyguanosin-8-yl)-ABP	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyguanosin-N^2-yl)-ABP	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyadenosin-8-yl)-ABP	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyguanosin-8-yl)-ABP	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyguanosin-N^2-yl)-ABP	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyadenosin-8-yl)-ABP	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyguanosin-8-yl)-ABP	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyguanosin-N_2-yl)-ABP	Radioisotope
4-Aminobiphenyl (ABP)	N-Hydroxy-ABP	N-(deoxyguanosin-8-yl)-ABP	Radioisotope
4-Acetylamino-4'-fluoribiphenyl	N-Hydroxy-AAFBP	N-(deoxyadenosin-8-yl)-AAFBP	
4-Acetylamino-4'-fluoribiphenyl	N-Hydroxy-AAFBP	3-(deoxyguanosin-N^2-yl)-AAFBP	
3,2'-Dimethyl-4-aminobiphenyl (DMABP)	N-Hydroxy-DMABP	N-(deoxyguanosin-8-yl-)-DMABP	Radioisotope
3,2'-Dimethyl-4-aminobiphenyl (DMABP)	N-Hydroxy-DMABP	5-(deoxyguanosin-N^2-yl)-DMABP	Radioisotope

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
40mg/kg(A); 2,4 fmol adduct/μg DNA	62% D D0.02-D1; 62% D D1-D29	Rat	Liver	15
40mg/kg(A); 1.5 fmol adduct μg DNA	80% D D0.02-D1; 100% D D1-D9	Rat	Liver	15
40mg/kg(A); 13.2 fmol adduct μg DNA	77% D D0.02-D1; 93% D D1-D29	Rat	Liver	15
N/A	N/A	In vitro	N/A	33
N/A	N/A	In vitro	N/A	33
N/A	N/A	In vitro	N/A	33
60μmol/kg; ~ 300-800 adducts/10^8 nucleotides	~ 130% I D1-D7	Dog	Liver	33
60μmol/kg; ~ 0CX<200 adducts/10^8 nucleotides	~ 0% D D1-D7	Dog	Liver	33
60μmol/kg; ~ 0CX<200 adducts/10^8 nucleotides	~ 0% D D1-D7	Dog	Liver	33
60μmol/kg; ~ 300-600 adducts/10^8 nucleotides	~ 100% I D1-D7	Dog	Bladder	33
60μmol/kg; ~ 0<X<100 adducts/10^8 nucleotides	~ 0% D D1-D7	Dog	Bladder	33
60μmol/kg; ~ 0<X<100 adducts/10^8 nucleotides	~ 0% D D1-D7	Dog	Bladder	33
	T 1/2 = 2D	Rat	Liver, kidney	29
		Rat	Liver, kidney	29
0.5 mmol/kg; ~ 60 pmol DMABP/mg DNA (est.)	~ 20% D D1-D7	Rat	Liver	229
0.5 mmol/kg; ~ 20 pmol DMABP/mg DNA (est.)	~ 20% D D1-D7	Rat	Liver	229

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
3,2'-Dimethyl-4-aminobiphenyl (DMABP)	N-Hydroxy-DMABP	N-(deoxyguanosin-8-yl)-DMABP	Radioisotope
3,2'-Dimethyl-4-aminobiphenyl (DMABP)	N-Hydroxy-DMABP	5-(deoxyguanosin-N^2-yl)-DMABP	Radioisotope
3,2'-Dimethyl-4-aminobiphenyl (DMABP)	N-hydroxy-DMABP	5-(deoxyguanosin-N^2-yl) DMABP, N-(deoxyguanosin-8-yl)-DMABP	
""	""	""	
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AAF	
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	3-(deoxyguanosin-N^2-8-yl)-AAF	
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AAF	
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AAF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	3-(deoxyguanosin-8-yl)-AAF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	(Guan-8-yl)-DNA Adducts	Immunoassay
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	(Guan-8-yl)-DNA Adducts	Immunoassay

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
0.5 mmol/kg; ~ 40 pmol DMABP/mg DNA (est.)	~ 70% D D1-D7	Rat	Internal mucosa	229
0.5 mmol/kg; ~ 10 pmol DMABP/mg DNA (est.)	~ 70% D D1-D7	Rat	Internal mucosa	229
	70% D D0-D7	Rat	Intestine	230
	29% D D0-D7	Rat	Intestine	230
	T 1/2 = 7D	Rat	Liver	123,124
		Rat	Liver	123,124
		Rat	Liver	29
10 mg/kg; biweekly to 6 weeks; 1-1.8 pmol AAF/mg DNA	100% D D1-D14	Rat (male)	Liver	31
10 mg/kg; biweekly to 6 weeks; 0.8-3.2 pmol AAF/mg DNA	20-90% D D1-D14	Rat (male)	Liver	31
10 mg/kg; biweekly to 6 weeks; 12-58 pmol AAF/mg DNA	10-50% D D1-D14	Rat (female)	Liver	31
10 mg/kg; biweekly to 6 weeks; 15-20 pmol AAF/mg DNA	50-80% D D1-D14	Rat (male)	Liver	31
10 mg/kg; biweekly to 6 weeks; 5-16 pmol AAF/mg DNA	10% 1-60% D D1-D14	Rat (female)	Kidney	31
10 mg/kg; biweekly to 6 weeks; 2-4 pmol AAF/mg DNA	~ 50-80% D D1-D14	Rat (male)	Kidney	31
0.02% in feed, up to 60 days; 80-238 fmol adduct/μg DNA	50% D D1-D28	Rat (male)	Liver	175
0.04% in feed, up to 46 days; 129-252 fmol adduct/μg DNA	90% D D1-D28	Rat (male)	Liver	175

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	(Guan-8-yl)-DNA Adducts	Immunoassay
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	(Guan-8-yl)-DNA Adducts	Immunoassay
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	(Guan-8-yl)-DNA Adducts	Immunoassay
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AF	Radioisotope
2-Acetylaminofluorene (AAF)	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-AF	Radioisotope
""	""	""	""
""	N-Hydroxy-AAF and N-Acetoxy AAF	"" and 3-(deoxyguanosin-N^2-yl)-2-AAF	Radioisotope
""	N-Hydroxy-AAF	N-(deoxyguanosin-8-yl)-2-AAF	""
""	""	"" and 3-(deoxyguanosin-N^2-yl)-AAF and AAF-N-7 adducts with guanosine	""
Benzidine (BZ)	N-Hydroxy-N'-acetyl-BZ	N-(deoxyguanosin-8-yl)-N'-acetyl-BZ	Radioisotope
Acetyl-BZ	N-Hydroxy-N'-acetyl-BZ	N-(deoxyguanosin-8-yl)-N'-acetyl-BZ	Radioisotope
Acetyl-BZ	N-Hydroxy-N'-acetyl-BZ	N-(deoxyguanosin-8-yl)-N'-acetyl-BZ	Radioisotope
Diacetyl-BZ	N-Hydroxy-N,N'-diacetyl-BZ	N-(deoxyguanosin-8-yl)-N,N'-diacetyl-BZ	Radioisotope

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
0.02% in feed, up to 60 days; 14-31 fmol adduct/µg DNA	45% D D1-D28	Rat (male)	Kidney	175
0.04% in feed, up to 60 days; 15-47 fmol adduct/µg DNA	50% D D1-D28	Rat (male)	Kidney	175
0.04% in feed, up to 60 days; 0-27 fmol adduct/µg DNA	65% D D1-D28	Rat (male)	Adrenal	175
60 µmol/kg; 500 adducts/10^8 nucleotides	80% D D2-D7	Dog	Liver	33
60 µmol/kg; 100 adducts/10^8 nucleotides	70% D D2-D7	Dog	Bladder	33
		Mouse	Liver	127
--	T 1/2 = 6D	Rat	Liver	124, 228
40 mg/kg; 1.5 adducts/10^6 nucleotides	5 1/2 = 14.2D	Rat	Mammary gland	6
--	N/A	In vitro	N/A	211
1-230 µM; -	--	Salmonella typhimurium	N/A	37
--	N/A	In vitro	N/A	214
80 ppm in water for 1 week; ~ 90 pmol BZ/mg DNA	50% D D0-D1; 60% D D0-D7	Rat	Liver	138
111 µmol/kg; 70 pmol/mg DNA	60% D D1-D7	Rat	Liver	229
111 µmol/kg; 33 pmol/mg DNA	60% D D1-D7	Hamster	Liver	229
111 µmol/kg; 5 pmol/mg DNA		Rat	Liver	229

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
N-Methyl-4-aminoazobenzene (MAB)	N-Sulfonyloxy-MAB	N-(deoxyguanosin-8-yl)-MAB	Radioisotope
N-Methyl-4-aminoazobenzene (MAB)	N-Sulfonyloxy-MAB	3-(deoxyguanosin-N^2-yl)-MAB	Radioisotope
N-Methyl-4-aminoazobenzene (MAB)	N-Sulfonyloxy-MAB	3-(deoxyadenosin-N^6-yl)-MAB	Radioisotope
N-Methyl-4-aminoazobenzene (MAB)	N-Sulfonyloxy-MAB	N-(deoxyguanosin-8-yl)-MAB	Radioisotope
N-Methyl-4-aminoazobenzene(MAB)	N-Sulfonyloxy-MAB	N-(deoxyguanosin-8-yl)-MAB, plus N-7 substituted adducts with guanine	Radioisotope
MAB	N-sulfonyloxy-MAB	3-(deoxyguanosinN^2-yl)-MAB	Radioisotope
4-Aminoazobenzene	N-Hydroxy-AB (?)	N-(deoxyguanosin-8-yl)-AB	Radioisotope
2-Acetylaminophenanthrene (AAP)	N-Hydroxy-AAP	N-(deoxyguanosin-8-ul)aminophenanthrene (AP)	^{32}P-Postlabeling
Benzo(a)pyrene (BaP)	(+)7,8-hydroxy-9,10-epoxy BaP (BPDEI)	--	Radioisotope
BaP	(-)7,8-hydroxy-9,10-epoxy BaP (BPDEII)	--	Radioisotope
BaP	BPDEI, BPDEII	N^7 adduct with guanine; adenine adducts, N-2 guanine add.	Radioisotope
BaP	BPDEI, BPDEII	--	Radioisotope
BaP	BPDEI, BPDEII	BBDEI-guanosine, BPDEI-protein	Radioisotope
BaP	--	--	Radioisotope
BaP	BPDEI, BPDEII	--	Radioisotope

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
0.2 mmol/kg; days 1,3,5,8 0.1-2 adducts/10^6 nucleotides		Rat	Liver	216
0.2 mmol/kg, days 1,3,5,8 0.1-1 adducts/10^6 nucleotides		Rat	Liver	216
0.2 mmol/kg, days 1,3,5,8 0-0.9 adducts/10^6 nucleotides		Rat	Liver	216
120 mg/kg; ~ 8 μmol adduct/mol DNA	T 1/2 ~ 5D	Mouse	Liver	213
	N/A	In vitro	N/A	214
120 mg/kg ~ 1.5 μmol adduct/mol DNA	T 1/2 between 5 and 10 D	Mouse	Liver	213
		Mouse (pre-weanling)	Liver	54
40 mg/kg; 27 fmol adduct/μg DNA	50% D1-D9	Rat	Liver	75
----	----	Human	Colon explants	39
----	----	Human	Colon explants	39
----	----	Numerous	Numerous	14, 203
Linear 0.01-300 μg/mouse	----	Mouse	Epidermis	163
Linear 2-11 μmol/kg sigmoidal 11-135 μmol/kg	----	Mouse	Lung, liver, forestomach	3
1 μmol/kg -- 10 μmol/kg -- 3-5 X increase	----	Rat	Lung, liver	39
incubated 1μM BaP 10-30X adduct level difference	----	Human, various others	Bladder, trachea	51

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
BaP	BPDEI, BPDEII	--	Fluorescence
BaP	BPDEI, BPDEII	Protein	Fluorescence
BaP	BPDEI, BPDEII	--	Radioisotope
BaP	BPDEI, BPDEII	BPDE-dG, BDDE-dA	Radioisotope
BaP	BPDEI, BPDEII	BPDE-dG, BDDE-dA	Radioisotope
BaP	BDEI	BDDEI-DNA	Radioisotope
BaP	BDEI	BDDEI-DNA	Radioisotope
Benzo(a)pyrene	BPDE	Protein adduct (Hemoglobin)	Fluorescence
""	BPDE-I	7R BPDE I-deoxyguanosine	Radioisotope
""	""	BPDE-N^2-deoxyguanosine and 9-hydroxybenzo(a)pyrene-DNA	Radioisotope
""	""	""	""
""	""	7R, 7S BPDE-N^2-deoxyguanosine	Radioisotope
""	""	""	""
""	""	""	""
""	""	""	""

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
Linear 50-500 µg/mouse	T 1/2 = ~2D, T 1/2 = ~ 30D	Mouse	Skin	195
Linear 50-500 µg/mouse	----	Mouse	Hemoglobin	195
200nmol/mouse ~7-15 pmol BPDE/mg macromolecule	T 1/2 ~ 2-3D (all)	Mouse	Skin, DNA, RNA, protein	162
250nmol/mouse 5.2 pmol BPDE/mg DNA	76% D D1-D7,93% D D1-D-21	Mouse	Skin	5
1000nmol/mouse 12.36 pmol BPDE/mg DNA	56% D D1-D7, 100% D D1-D21	Rat	Skin	5
6 mg BaP/mouse 6, 7 pmol BPDEI/mg DNA (Lung, Liver)	T 1/2 (lung 20D, liver 14D)	A/HeJ mouse	Lung, liver	125
6 mg BaP/mouse 6, 3 pmol BPDEI/mg DNA (lung, liver)	T 1/2 (lung 15D, liver 3D)	C57BL/6J mouse	Lung, liver	125
	T 1/2 = 6D	Mouse	Hemoglobin	194
	95% D D0-D21	Mouse	Skin	4
200 nmol/mouse; 3.3 pmoles/mg DNA	--	Swiss mouse	Skin	11
200 nmol/rat; 0.74 pmoles/mg DNA	33% D H12-H48	Rat	Skin	12
1.5 µM BPDEI; 15 µmol/mol DNA	56% D H0=H8, 67% D H0-H24	Human	Fibroblasts	111
--; 14.2 pmol/mg DNA	40% D D1-D3 (total adducts)	Rat	Embryo cells	23
--; 6.0 pmol/mg DNA	47% D D1-D3 (total adducts)	Hamster	Embryo cells	23
--; 4.0 pmol/mg DNA	30% D D1-D3 (total adducts)	Human	Hepatoma cells	23

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
""	--	--	^{32}P postlabeling
""	""	9-OH-BP-4,5-epoxided-dG, 7S, 7R-BPDEL-dG, and 7R-BPDE II-dG	Radioisotope
""	""	Trans-7R-BPDEI-N^2-deoxyguanosine	Immunoassay
10-Azabenzo(a)pyrene-4,5-oxide	Direct acting		
9-Anthroyloxirane (9-AO)	Direct acting	N-3 adduct of 9-AO to adenine	UV-absorbance
1-Oxiranylpyrene	Direct acting	Similar to BaP (?)	UV-absorbance
3-Methylcholanthrene (3-MC)	3-MC Diolepoxide	----	----
3 MC	3-MC Diolepoxide	----	Radioisotope
3 MC	3-MC Diolepoxide	----	Radioisotope
3 MC	3-MC Diolepoxide	----	Radioisotope
3 MC	3-MC Diolepoxide	----	Radioisotope
3 MC	3-MC Diolepoxide	----	Radioisotope
3 MC	3-MC Diolepoxide	----	Radioisotope
3 MC	3-MC Diolepoxide	----	Radioisotope
3 MC	3-MC Diolepoxide	----	Radioisotope

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
4 doses 1.2 μmol/mouse; 1 adduct 6.2 x 10^4 cells	1 D < T 1/2 < 6D	Mouse	Skin	178
5.5 μM BaP; (0.75, 0.33, 3.0, 1.34) x 10^7 moles	61-73% D D0-D2	Human	Fibroblasts	110
Adducts/mol DNA				
Nonlinear 50-1500 nmol/ 2.3-11 fmol μg DNA	T 1/2 = 3D	Mouse	Skin	154
				157
----	N/A	In vitro	N/A	237
----	N/A	In vitro	N/A	117
----	----	Human	Bronchus, colon, esophagus	77
31 nmol 3-MC 45 fmol adduct/mg DNA	62% D D0-D28	A/J mouse	Lung	203
31 nmol 3-MC 5.5 fmol adduct/mg DNA	100% D D0-D28	A/J mouse	Liver	203
31 nmol 3-MC 18 fmol adduct/mg DNA	38% D D0-D28	C3H/HeJ mouse	Lung	203
31 nmol 3-MC 4.4 fmol adduct/mg DNA	100% D D0-D28	C3H/HeJ mouse	Liver	203
31 nmol 3-MC 16 fmol adduct/mg DNA	81% D D0-D28	DBA/2J mouse	Lung	203
31 nmol 3-MC 4.8 fmol adduct/mg DNA	100% D D0-D28	DBA/2J mouse	Liver	203
31 nmol 3-MC 16 fmol adduct/mg DNA	77% D D0-D28	C57BL/6J mouse	Lung	203
31 nmol 3-MC 9.7 fmol adduct/mg DNA	100% D D0-D28	C57BL/6J mouse	Liver	203

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
7,12-Dimethylbenz[a]anthracene (DMBA)	DMBA 3,4-diol-1,2-epoxide, 7-hydroxymethyl-12-methylbenz(a)anthracene diol-epoxide	DBMA-DNA	Radioisotope
7,12-Dimethylbenz[a]anthracene (DMBA)	"	DBMA-DNA	Radioisotope
7,12-Dimethylbenz[a]anthracene (DMBA)	"	DBMA-DNA	Radioisotope
7,12-Dimethylbenz[a]anthracene (DMBA)	"	DBMA-DNA	Radioisotope
7,12-Dimethylbenz[a]anthracene (DMBA)	Syn-DMBA-diol-epoxide	Syn-DMBA diol-epoxide-dAdo	Radioisotope
7,12-Dimethylbenz[a]anthracene (DMBA)	Anti DMBA diol-epoxide	Anti DBMA diol-epoxide-dAdo, dGuo	Radioisotope
15,16-Dihydro-11-methylcyclopenta[a]phenanthrene-17-one(11-methylketone)	1,2-epoxy-3,4-dihydroxy-11-methyl ketone	11-methyl ketone diol-epoxide-DNA	Radioisotope
11-methylketone	1,2-epoxy-3,4-dihydroxy-11-methyl ketone	11-methyl ketone diol-epoxide-DNA	Radioisotope
11-methylketone	1,2-epoxy-3,4-dihydroxy-11-methyl ketone	11-methyl ketone diol-epoxide-DNA	Radioisotope
11-methylketone	1,2-epoxy-3,4-dihydroxy-11-methyl ketone	N^2 guanine adduct with 11-methylketone diol-epoxide	Absorbance MS
11-methylketone	1,2-epoxy-3,4-dihydroxy-11-methyl ketone	----	Radioisotope
11-methylketone	1,2-epoxy-3,4-dihydroxy-11-methyl ketone	----	Radioisotope
7-Bromomethyl benzanthracene (7-BMBA)	----	----	Radioisotope

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
20, μmol/kg; 69 μmol DMBA/mol DNA	73% D D2-D14	Long-Evans rat	Liver	50
20 μmol/kg; 53.3 μmol/DMBA/mol DNA	31% D D2-D14	Long-Evans rat	Mammary gland	50
20 μmol/kg; 37.8 μmol DMBA/mol DNA	26% D D2-D14	Sprague-Dawley rat	Liver	50
20 μmol/kg; 28.6 μmol DMBA/mol DNA	7% D D7-D14	Sprague-Dawley rat	Mammary gland	50
0.01 μmol/mouse -- 0.1 μmol/mouse -- 3-4XI	11-25% D D1-D2	NIH-Swiss, C55BL mice	Skin	59
0.01 μmol/mouse -- 0.1 μmol/mouse -- 3-4XI	2-34% D D1-D2	NIH-Swiss, C55BL mice	Skin	59
----	N/A	In Vitro	N/A	1, 203
----	----	Mouse	Skin	1, 203
----	----	Mouse	Embryo cells	203
----	N/A	In Vitro	N/A	231
3 mg/mouse; 283, 345 nmol adduct/mol DNA	T 1/2 ~ 6.5 D	Mouse	Skin, lung	2
3 mg/mouse, 641 nmol adduct/mol DNA	T 1/2 ~ 2.5 D	Mouse	Liver	24
7 nmol/mouse, 44 adducts/10^7 nucleotides	T 1/2 < 1 D	Mouse	Liver	24

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
7-Bromomethyl benzanthracene (7-BMBA)	----	----	Radioisotope
7-Bromomethyl benzanthracene (7-BMBA)	----	----	Radioisotope
1-Nitropyrene (1-NP)	N-hydroxy-1-aminopyrene	N-(deoxyguanosin-8-yl)-1-aminopyrene	Radioisotope
1-Nitropyrene (1-NP)	N-hydroxy-1-aminopyrene	----	Radioisotope
1-Nitropyrene (1-NP)	N-hydroxy-1-aminopyrene	N-(deoxyguanosin-8-yl)-1-aminopyrene	Radioisotope
1,8-dinitropyrene	1-amino-8-nitropyrene	N-(deoxyguanosin-8-yl)-1-amino-8-nitropyrene	Radioisotope
Dibenzo(a,e)fluoranthene (DBF)	3,4-dihydroxy-1,2-epoxy-DBF	N^2-adduct with guanine	MS
Dibenzo(a,e)fluoranthene (DBF)	12,13-dihydroxy-11,2-epoxy-DBF	N^2-adduct with guanine	MS
Dibenzo(a,e)fluoranthene (DBF)	12,13-dihydroxy-11,2-epoxy-DBF	----	Radioisotope
5-methylchrysene (5-MC)	1,2-dihydroxy-3,4-epoxy-5MC (DEII) and	N^2-guanosine adducts with DE-I and DE-II	Radioisotope
	7,8-dihydroxy-9,10-epoxy-5MC (DEII)		
5-MC	DEI, DEII		Radioisotope
4-Nitroquinoline-1-oxide	O-acetyl,O,O'-diacetyl-	N-(deoxyguanosin-8-yl)-4NQO	--------
(4NQO)	4-hydroxyaminoquinoline		
4-Nitroquinoline-1-oxide	O-acetyl,O,O'-diacetyl-	N-(deoxyguanosin-8-yl)-4NQO	Radioisotope
(4NQO)	4-hydroxyaminoquinoline		

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
1 nmol/mouse, 48 adducts/10^7 molecules	T 1/2 ~ 15 D	Mouse	Liver histone	24
1 nmol/mouse, 1400 adducts/10^6 molecules	T 1/2 ~ 1 D	mouse	Liver albumin	24
	--	Salmonella typhimurium	N/A	87, 96
100 µM1-NP, 103 pmol 1-NP/mg DNA	N/A	In Vitro	N/A	25
8.1 µM 1-1NP; ----	--	Rabbit	Lung	98
3 µM 1-amino-8-nitropyrene; ----	--	Salmonella typhimurium		96,87
--	N/A	In Vitro	N/A	166
--	N/A	In Vitro	N/A	166
--	50% D D0-D2	Mouse embryo	Fibroblasts	167
--	--	In Vitro	N/A	144
-				144
10 nmol/mouse, ~ 3 pmol adducts/mg DNA	20% D D0-D2	Mouse	Skin	142, 143
--	--	In vitro	N/A	21, 67
--	unclear	Rat	Pancreas, liver, kidney, lung blood	56

OMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
flatoxin B, (AFB)	--	8,9-dihydro-8-(N^5-formyl-2',5',6'-triamino-4'-Oxo-N^5-pyrimidyl)-9-hydroxy AFB_1, and 8,9-Dihydro-8-(N^7-guanyl)-9-hydroxy AFB,	Radiosiotope
flatoxin B, (AFB)	--	""	Radioisotope
flatoxin B, (AFB)	AFB -2,3-epoxide	2,3-dihydro-2-(N^5-formyl)-2',5',6'-triamino-4'-Oxo-N^5-pyrimidyl)-3-hydroxyaflatoxinB_1, and 2,3-dihydro-2-(N^7-guanyl)-3-hydroxyaflatoxin B_1	Radioisotope
Aflatoxin B, (AFB)	AFB -2,3-epoxide	""	-
Aflatoxin B, (AFB)	AFB -2,3-epoxide	""	Radioisotope
Aflatoxin B, (AFB)	AFB -2,3-epoxide	2,3-dihydro-2-(N^7-guanyl)-3-hydroxyaflatoxin B_1	Absorbance
Sterigmatocystin (STO)	ST-1,2-epoxide	ST-N-guanine adduct	^{32}post-labeling
Mitmycin C (MMC)	MMC-reduction product	N^2-gua-,O^6-gua-, and N^6-Ado-MMC	Absorbance
3-amino-1-methyl-5H-pyrido[4,3-b]	N-OH-Trp-P-2	3-(C^8-guanyl)amino-1-methyl-5H-pyrido[4,3-b] Indole (Gua-Trp-P-2)	Absorbance
2-amino-6-methyl-dipyrido [1,2-a:3',2'-d]imidazole (Glu-P-1)	N-OH-Glu-P-1	2-(C^8-guanyl)amino-60methyldipyrido[1,2-a:3', 2'-d]imidazole (Gua-Glu-P-1)	Absorbance

OSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
1 µM AFB, 2.2 -- 135 µ mol AFB/mol DNA	--	Human, rat, dog, hamster	Trachea, or bronchial tissues	202
1 µM AFB, 1.5 -- 26 µmol AFB/mol DNA	--	""	Bladder tissues	202
.3 µM, 5-8 µmol adduct/mol DNA	T1/2 ~ 12 H	Mouse	Embryo fibroblasts	8
--	N/A	In vitro	N/A	49
0.6 mg/kg; 268 pmol/mg DNA	T 1/2 (> 72H, 7.5H)	Rat	Liver	48
Linear 0.125 -- 0.5 mg/kg; sigmoidal 10.125 10 mg/kg	N/A	Rat	Urine	35
Linear 1-9 mg/kg , 1 ogarhythmic 0.33-9 mg/kg	T 1/2 12 h, 7D, 109D	Rat	Liver	183
	N/A	In vitro	N/A	84
--	N/A	In vitro	N/A	82, 85
--	N/A	In vitro	N/A	82, 85

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
3-amino-4,6-dimethylpyrido[1,2-a: 3',2-d]imidazole(aGlu-P-3)	N-AcO-AGlu-P-5	Glu-P-3 bound to C^8 of guanine	
Safrole	1'-Hydroxysafrole-derivations	N^2(trans-isosafrol-3'-yl)deoxyguanosine and N^6-(trans-isosafrol-3'-yl)deoxyadenosin	Radioisotope
Safrole	1'-Hydroxysafrole-derivations	""	Radioisotope
Safrole	1'-Hydroxysafrole-derivations	""	Radioisotope
Safrole	1'Hydroxysafrole-derivations	The 2 adducts above plus 8-(trans-isosafrol-3'-yl)- and 7-(trans-isosafrol-3'-yl)guanine	Radioisotope
Estragole	1'-Hydroxyestragole-derivations	N^2-(estragol-1'-yl)deoxyguanosin,N^2-(cis-isoestragol-3'-yl)deoxyguanosine, trans-isoestragol-3'-yl)deoxyguanosine, and N^6-(trans-isoestragol-3'-yl)deoxyadenosine	Radioisotope
Estragole	1'-Hydroxyestragole-derivations	""	Radioisotope
Estragole	1'-Hydroxyestragole-derivations	""	Radioisotope
Estragole	--	--	^{32}P-post labeling
Allylbenzene	--	--	""

)OSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
				136
12 µmol/mouse 350 pn. [illegible]g DNA	T 1/2 ~ 2D	Mouse	Liver	170
12 µmol/mouse, 275 pmol/mg tRNA	T 1/2 ~ 2D	Mouse	Liver tRNA	170
12 µmol/mouse, 250 pmol/mg protein	T 1/2 ~ 2D	Mouse	Liver protein	170
--	--	Mouse	Liver	234
12 µmol/mouse, 250 pmol adduct/mg DNA	T 1/2 ~ 4D (total adducts)	Mouse	Liver	169
12 µmol/mouse; 200 pmol adduct/mg tRNA	T 1/2 ~ 2.5D (total adducts)	Mouse	Liver tRNA	169
12 µmol/mouse; 250 pmol adduct/mg protein	T 1/2 ~ 2D (total adducts)	Mouse	Liver protein	169
10 mg/mouse; ~ 290 pmol adduct/mg DNA	Persistent after 43 D	Mouse	Liver protein	171, 179
10 mg/mouse; 3.1 pmol adduct/mg DNA	--	Mouse	Liver protein	171, 179

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
Safrole	--	--	""
Myristicin	--	--	^{32}P-postlabeling
Dill apiol	--	--	""
Parsley apiol	--	--	""
Isosafrole	--	--	""
Methyleugenol	--	--	""
Elemicin	--	--	""
Anethole	--	--	""
Trimethylpsoralen	UV activated	Linked to 5,6 positions of thymine (cycloadduct)	--
8-methoxypsoralen	UV activated	""	--
Angelicin	UV activated	""	--
5-methylisopsoralen	UV activated	""	--
Monocrotaline (MC)	Pyrrole form of MC	7 position of MC bound to N^2 position of deoxyguanosine	--
CC-1065	--	bound to N^3 position of adenine	--
Cis-diamminedichloroplatinum (II)	Direct acting	Intra, interstrand links to N^7 of guanine; DNA protein crosslinks	Immunoassay
""	""	""	AA

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
10 mg/mouse; 205 pmol adduct/mg DNA	Persistent after 43D	Mouse	Liver, protein	171, 179
10 mg/mouse 50 pmol/mg DNA	Persistent after 43D	Mouse	Liver	171, 179
10 mg/mouse 40 pmol/mg DNA	Persistent after 43D	Mouse	Liver	171, 179
10 mg/mouse 14 pmol/mg DNA	--	Mouse	Liver	171, 179
10 mg/mouse 4.5 pmol/mg DNA	--	Mouse	Liver	171, 179
10 mg/mouse 196 pmol/mg DNA	Persistent after 43D	Mouse	Liver	171, 179
10 mg/mouse 16 pmol/mg DNA	Persistent after 43D	Mouse	Liver	171, 179
10 mg/mouse 1.3 pmol/mg DNA	--	Mouse	Liver	171, 179
--	--	--	--	44
--	--	--	--	44
--	--	--	--	44
--	--	--	--	44
--	--	--	--	185
--	--	--	--	206
Linear, 0-600 mg/M^2, 0-200 attomol/µg DNA	--	Man	White blood cells	177
--	T 1/2 6 min-45D	Various	Various	185a

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
""	""	""	AA
Cis-diamminetetrachloroplatinum (IV)	""	""	AA
Trans-diamminedichloroplatium	""	""	AA
Trans-diamminetetrachloroplatinum	""	""	AA
Diethylstilbestrol	--	--	^{32}P postlabeling
Promethazine (PMZ)	Radical cation or photoactivated	--	Radioisotope
Chlorpromazine (CPZ)	""	--	""
Promethazine, Chlorpromazine	""	--	""
Acrylonitrile	Direct acting	1-(2-carboxyethyl)-adenine, N^6-(2-carboxyethyl)-	MS
		Adenine, 3-(2-carboxethyl)-cytosine,7-(2-cyano-ethyl)-	
		Quanine, 7,9-bis(2-cyanoethyl)guanine, imidazole	
		Ring-opened 7,9-bis-(2-cyanoethyl)guanine, and	
		3-(2-cyanoethyl)-thymine	
Styrene	Styrene 7,8-oxide	N^7-(2-hydroxy-1-phenylethyl)guanine and	Radioisotope
		N^7-(2-hydroxy-2-phenylethyl)guanine	""
Styrene	""	--	""

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
Linear 25-200 mm, 0.8-2.5 crosslinks/10^9 daltons	T 1/2 ~ 24 H	Chinese hamster	Cultured ovary cells	173
""	""	""	""	173
""	""	""	""	173
Linear 100-800 μM 0.7-2.2 crosslinks/10 ^{9}daltons	--	""	""	173
DES treatment for 8 months	--	Hamster	Kidney	133
Linear 0-0.7 mM; 0-0.5% nucleosides adducted	--	In vitro; salmonella typhimurin	N/A	55
Linear 0-0.08 mM; 0-3% nucleosides adducted	--	""	N/A	55
Linear 0-0.03 mM; 0-40 nmol PMZ/mg protein	--	""	Protein	55
--	--	In vitro		201
Non-linear 1.1-4.9 mmol/kg; 17-31 nmol/g DNA	--	Mouse	Liver	41
Non-linear 1.1-4.9 mmol/kg; 3-60 nmol/g Hb	--	Mouse	Liver	41

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
" "	" "	--	" "
Styrene 7,8 oxide	Direct acting	Same as styrene	" "
" "	" "	--	" "
" "	" "	--	" "
Ethylene oxide	" "	--	" "
Ethylene oxide	" "	--	" "
Styrene 7,8 oxide	" "	--	" "
" "	" "	--	" "
" "	" "	--	" "
" "	" "	--	" "
Hydroxylamine	Direct acting	N^4-Hydroxycytosine	--
Dimethylcarbamyl chloride	Direct acting	6-dimethylcarbamyloxy-2'-deoxyguanosine 6-dimethylamin-2'-deoxyguanosine, and 4-dimethylaminothymidine	MS
Diethylcarbamyl chloride	Direct acting	6-diethylcarbamyloxy-2'-deoxyguanosine	MS
Trans-4-acetylaminostilbene	--	--	Radioisotope

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
Non-liner 1.1-4.9 mmol/kg; 200-430 mmol/g protein	--	Mouse	Liver	41
1.1 mmol/kg; 8 nmol/g DNA	--	Mouse	Liver	41
Non-linear 0.037-1.1 mmol/kg; 0.1-13 nmol/g Hb	--	Mouse	Liver	41
Non-linear 0.037-1.1 mmol/kg 9.8-750 nmol/g protein	--	Mouse	Liver	41
0.044 nmol/kg; 2 nmol/g DNA	--	Mouse	Liver	41
0.044 nmol/kg 2 nmol/g Hb	--	Mouse	Liver	41
0.36 mmol/kg; 5 nmol adducts/g DNA	--	Mouse	Brain	41
0.36 mmol/kg; 3 nmol adducts/g DNA	--	Mouse	Lung	41
0.36 mmol/kg; 0.6 nmol adducts/g DNA	--	Mouse	Spleen	41
0.36 mmol/kg; 0.3 nmol adducts/g DNA	--	Mouse	Testis	41
--	--	--	--	100
--	--	--	--	191
--	--	--	--	191
5 µmol/kg; twice weekly for 6 weeks; 24 pmol/mg DNA	T 1/2 = 22D	Rat	Liver	93

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
""	--	--	""
""	--	--	""
""	--	--	""
""	--	--	""
""	--	--	""
""	--	--	""
""	--	--	""
Trans-4-acetylaminostilbene	--	--	
Trans-4-acetylaminostilbene	--	--	
Trans-4-acetylaminostilbene	--	--	
Trans-4-acetylaminostilbene	--	--	
1-bromo-2-chloroethane	S-(2-bromoethyl)GSH or S-(2-bromoethyl)GSH	S-[2-(N^7-guanyl)ethyl]GSH	
N-butylmethanesulfonate	Direct acting	O^6-n-butyl-guanosine, N^7-n-butylguanosine and N^3-n-butyladenosine	Radioisotope
N-n-butyl-N-nitrosourea	Direct acting	O^6-n- and sec-butylguanosine, N^7-n- and sec-butylguanosine and 3-n-butyladenosine	Radioisotope

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
5 µmol/kg; twice weekly for 6 weeks; 18 pmol/mg DNA	Persistent for 6 weeks	Rat	Kidney	93
5 µmol/kg; twice weekly for 6 weeks; 2 pmol/mg DNA	T 1/2 = 18D	Rat	Lung	93
5 µmol/kg; twice weekly for 6 weeks; 3 pmol/mg DNA	T 1/2 = 9.0D	Rat	Gland stomach	93
5 µmol/kg; twice weekly for 6 weeks; 27 pmol/kg protein	T 1/2 = 5.1D	Rat	Liver, protein	93
5 µmol/kg; twice weekly for 6 weeks; 10 pmol/kg protein	T 1/2 = 8.7D	Rat	Kidney, protein	93
5 µmol/kg; twice weekly for 6 weeks; 4 pmol/kg protein	T 1/2 = 7.3D	Rat	Lung, protein	93
5 µmol/kg; twice weekly for 6 weeks; 7 pmol/kg protein	T 1/2 = 6.5D	Rat	Gland stomach, protein	93
5 µmol/kg; twice weekly for 6 weeks; 2 pmol/mg RNA	T 1/2 = 4.5D	Rat	Liver	93
5 µmol/kg; twice weekly for 6 weeks; 16 pmol/kg RNA	Persistent over 6 weeks	Rat	Kidney	93
5 µmol/kg; twice weekly for 6 weeks; 1 pmol/kg RNA	T 1/2 = 20.0D	Rat	Lung	93
5 µmol/kg; twice weekly for 6 weeks; 1 pmol/kg RNA	T 1/2 = 5.0D	Rat	Gland stomach	93
2 mM; 217 pmol/mg DNA	--	In vitro	N/A	160
Linear 1-10 mM; @ 10mM 4.1, 188, 10.6 µmol/mol DNA	--	In vitro	N/A	187
Linear 1-10 mM; @ 10mM 99, 29, 151, 17, and 155 µmmol/DNA	--	In vitro	N/A	187

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
1-(2-chloroethyl)-3-(cis-2-hydroxy) cyclohexyl-1-nitrosourea	Direct acting	7-hydroxyethyl aguanine and 7-chloroethyl guanine	Radioisotope
1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea	Direct acting	""	Radioisotope
1-chloroethyl(methyl sulfonyl)-methanesulfonate	Direct acting	""	Radioisotope
2-chloroethyl(methyl sulfonyl)-methanesulfonate	Direct acting	7-chloroethylguanine	
1-(2-chloroethyl)-1-nitrosourea	Direct acting	""	Radioisotope
1,2-Dibromoethane	S-(2-bromoethyl)GSH	S-[2-(N^7-guanyl)ethyl]GSH	Radioisotope
Diethylsulfate	Direct acting	$N^{1,3,7}$ethyladenine, $N^{3,7}$-guanine, N^3-cytosine	Radioisotope
Dichlorvos	Direct acting	N^7-methylguanine and N^3-methyladenine	Radioisotope
Diethylnitrosamine (DEN)	Direct acting	N^7, N^3-ethylguanine and N^3-ethyladenine	Radioisotope
" "	Direct acting	O^4-ethylthymidine	Radioisotope
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	N^7-methylguanine and O^6-methylguanine	Absorbance
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	""	""

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
	--	In vitro	N/A	38,80
	--	In vitro	N/A	38,80
	--	In vitro	N/A	38,80
	N/A	In vitro	N/A	38,80
	--	In vitro	N/A	38,80
2 mM; 618 pmol/mg DNA	--	In vitro	N/A	160
	--	In vitro	N/A	145, 196
--	--	Rat	Combined organs	236
50 mg/kg; 31, 3, 4 μmol/mol DNA	T 1/2 ~ 5D	Rat	Liver	57
40 ppm DEN in H_2O (8D) 5.0 pmol/μmol dT	T 1/2 ~ 11D	Rat	Liver	184
50 ppm SDMH in H_2O (28D) 700, 15 pmol/mg DNA	--	Rat	Non parenchymal cells	27
50 ppm SDMH in H_2O (28D) 800, 1 pmol/mg DNA	--	Rat	Hepatocytes	27

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	O^4-methylthymidine, O^6-methylguanine	Radioisotope
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	5 adducts	^{32}P postlabeling
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	N^7-,O^6-methylguanine	Radioisotope
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	""	Radioisotope
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	O^6-methylguanine	Fluorescence
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	N^7-methylguanine	Fluorescence
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	N^7-methylguanine	Fluorescence
1,2-Dimethylhydrazine (SDMH)	Methyldiazonium ion	7-Methylguanine, O^6-methylguanine	Absorbance
""	""	""	""
Dimethylnitrosamine	Direct acting	N^7-,O^6-methylguanine	^{32}P postlabeling
Dimethylnitrosamine	Direct acting	N^7, N^3, O^6-methyguanine, N^3, N^7-methyladenine	Radioisotope
Dimethylsulfate	Direct acting	N^7,O^6methylguanine, N^3-methyladenine	Radioisotope
Dimethylsulfate	Direct acting	N^7,N^3,O^6-methylguanine, N^7,N^1,N^3-methyladeine	Radioisotope
Dimethylsulfate	Direct acting	N^7,O^6-methylguanine, N^3-methyladenine	Radioisotope
Dimethylsulfate	Direct acting	N^3,N^7,O^6-methylguanine,N^1,N^3,N^7-methyladenine	Radioisotope
Dinitrosopiperazine	Direct acting	Yes	Radioisotope

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
20 mg/kg; 3.54, 494 pmol/mg DNA	T 1/2 ~ 30h, ~ 40 hr	Rat	Liver	184
?; one adduct/<10^4 nucleotides	--	Mouse	Liver	182
20 mg/kg; 9.1-34.8 µmol/mol guanine (0^6M Gua)	--	Mouse (ICR/Ha)	Intestine	99
20 mg/kg; 8.2-23.0 µmol/mol guanine (0^6M Gua)	--	Mouse (C57BL/Ha)	Intestine	99
21 mg/kg; weekly for 14 weeks, 5-40 µmol/mol Gua	--	Mouse	Kidney	91
21 mg/kg; weekly for 14 weeks, 200-1100 µmol Gua	--	Mouse	Liver	91
21 mg/kg; weekly for 14 weeks, ~ 30-70 µmol/mol Gua	--	Mouse	Kidney	91
30 ppm, in H_2O; 1000, 50 pmol/mg DNA	T 1/2 > 30 D ~ 15D	Rat	Nonparenchymal cells	28
"" ; 1200, 30 pmol/ng DNA	T 1/2 > 30 D ~ 2D	Rat	Hepatocytes	28
150 mg/kg; one adduct/<10^4 nucleotides	--	Mouse	Liver	182
10 mg/kg; 379, 2, 37, 9, ~1 µmol/mol DNA	1D < T 1/2 < 6D	Rat	Liver	57
0.08mM; 86, 10 µmol/mol DNA	T 1/2 ~18, 3h	V79 cell	N/A	46
?; 3, 79, 1, 0.4, 0.4, 0.6, 16, µmol/mol DNA	--	Balb C mouse	Spleen cells	79
15 µg/ml 92, 0.5, 12, µmol/mol DNA	--	V79 cell	N/A	156
--	--	In vitro	N/A	156
-	--	Human	Bronchus, colon cells	77

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
Epichlorohydrin	Direct acting	N^7,O^6-alkylguanine	Radioisotope
Ethionine	?	N^7-ethyl guanine	Radioisotope
Ethionine	?	""	""
Ethionine	?	""	""
Ethylmethanesulfonate	Direct acting	N^7-O^6-methylguanine	--
Ethylmethanesulfonate	Direct acting	N^7,N^3-methylguanine,N^7,N^3-ethyladenine	Radioisotope
Ethylmethanesulfonate	Direct acting	Ethylated DNA	""
Ethylmethanesulfonate	Direct acting	""	""
Ethylnitrosoguanidine	Direct acting	N^1,N^3,N^7-ethyladenine,N^3,N^7-ethylguanine,	--
		N^3-ethyl-cytosine, and N^3-ethyl(uridine or thymine)	
Ethylnitrosourea	Direct acting	N^1,N^3,N^7-ethyladenine,N^3,N^7-ethylguanine,	--
		N^3-ethyl-cytosine, and N^3ethyl(uridine or thyamine)	
Ethylnitrosourea	Direct acting	N^7,O^6-ethylguanine,O^2-cytosine,O^2,O^4thymine	Radioisotope
Ethylnitrosourea	Direct acting	N^7-ethylguanine,O^2-ethylthymine, and O^2-ethylcytosine	""
Ethylnitrosourea	Direct acting	O^6-ethylguanine	Immunoassay
Ethylnitrosourea	Direct acting	""	Immunoassay

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
--	--	Rodents	--	26
Nonlinear 31-500 mg/kg; 120-3800 pmol/mg DNA	--	Rat	Liver	130
Nonlinear 31-500 mg/kg; 40-850 pmol/mg RNA	--	Rat	Liver	130
Nonlinear 31-500 mg/kg; 1100-10000 pmol/mol protein	--	Rat	Liver	130
--	--	Rat	--	26
200 mg/kg; 48, 1, ~ 1, 2, µmol/mol DNA	--	Rat	Liver	57
Nonlinear 2.5-50mM 0.8-8.6 ethylations/10^4 nucleotides	--	Yeast (neurospora crassa)	N/A	221
Nonlinear 2.5-50mM 0.6-6.7 ethylations/10^4 nucleotides	--	Yeast (saccharomyces cerevisiae)	N/A	221
--	--	In vitro	N/A	196
--	--	In vitro	N/A	196
	--	Mammalian cells	N/A	196
--	T 1/2 30-55 hr	Rat	Liver	196
--	T 1/2 40, 50, 60 hrs	In vitro	N/A	153
Linear 0.1-200 µg/ml adduct $3x10^7$ dGuo to 1 adduct/$7.9x10^3$ dGuo	--	Rat	--	--
Linear 2.5-100 µg/g 1 adduct/$2x10^5$ dGuo to 1 adduct/$4x10^4$ dGuo	--	--	Liver, brain	153

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
Ethylnitrosourea	Direct acting	N^3-ethyladenine, N^7ethylguanine, O^6-ethylguanine	Radioisotope
Ethylnitrosourea	Direct acting	O^6-ethylguanine	Radioisotope
""	""	N^7-ethylguanine	""
1-(2-Fluoroethyl)-3-cydohexyl-1-nitrosourea	Direct acting	7-hydroxy ethylguanine and 7-chloroethylguanine	Radioisotope
Glycidaldehyde	Direct acting	N^7,O^6-methylguanine	--
Gyromitrin	Decomposition products	N^7-methylguanine	Radioisotope
Hydrazine	Tetraformyltrisazine	O^6,N^7-methylguanine	
Methylmethane sulfonate	Direct acting	N^7-methylguanine	MS
Methylmethane sulfonate	Direct acting	""	""
Methylmethane sulfonate	Direct acting	N^7,O^6-methylguanine	--
Methylmethane sulfonate	Direct acting	N^3-methyladenine and N^7-methylguanine	--
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone	4-(methylnitrosamino)-1-(3-pyridyl)-1-butan-1-ol	O^6-methylguanine	Immunoassay
""	""	""	""
""	""	""	""

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
--	78%D, 34%D, 5%HO-H22	Chinese hamster ovary cells	--	71
10 mg/kg; 1.31 adducts/10^6 guanine residues	1D < T 1/2 < 3D	Mouse	Testis	190
"" ; 1.79 adducts/10^6 guanine residues	1 D < T 1/2 < 3D	""	""	190
--	--	In vitro	N/A	80
--	--	Rat	--	26
--	--	Rat	Liver, lung	86
		Rat	Liver	128
50 mg/kg; 12 μg/24 hr	N/A	Rat	Urine	65
50 mg/kg; 22 ng/10 mg globin	--	Rat	Globin	65
--	--	Rat	--	26
--	T 1/2 2.5, 11 H	Chlamydomonas	N/A	207
482 nmol/ml (1,3,24 hr culture time); 39, 65, and 138 μmol/mole guanine	--	Cultured rat nasal mucosa		42
87 mg/kg; 219,13,34 μmol/mole guanine	--	Rat	Nasal mucosa, lung liver	42
40 mg/kg; daily for 14D; 8,11 μmol/mole guanine	--	Rat	Nasal mucosa, lung	42

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
N-methyl-N-nitrosourea	Direct acting	N^7,O^6-methylguanine,3-methyladenine	Radioisotope
""	Direct acting	N^7,$N^3$$O^6$-methylguanine,$N^1$-$N^3$-methyl adenine O^4-methylthimine,ring opened N^7-methylguanine	Radioisotope
""	Direct acting	N^7-methylguanine	MS
""	Direct acting	""	""
""	Direct acting	--	^{32}P-postlabeling
""	Direct acting	N^3,N^7,O^6methylguanine$N^1$$N^3$$N^7$-methyladenine	Radioisotope
""	Direct acting	""	Radioisotope
N-methy-N-nitrosourea	Direct acting	N^7,O^6-methylguanine,N^3-methyladenine	Radioisotope
Metrifonate	Direct acting (?)	N^7-methylguanine	Radioisotope
Metrifonate	Direct acting (?)	""	Radioisotope
Metrifonate	Direct acting (?)	N^7,O^6-methylguanine	Radioisotope
Mitozolomide	Direct acting (?)	N^7-chloroethylguanine and N^7-hydroxyethylguanine	
N-Nitroso-N-acetoxymethyl-N 2-oxopropylamine	2-oxopropyldiazotate	O^6,N^7-methylguanine and N^3-methyladenine	UV absorbance
1-Nitroso-5,6-dihydrouracil	2-carboxyethylcarbonium ion	7-(2'-carboxyethyl) guanine	--

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
8.7 μg/ml;?	55%D, 2%I, 84%D	Chinese hamster ovary cells		71
0.5 mg/rat; 252, 2, 29, 1, 13, 6.5, 17 adducts	HO-H2O	Rat	Urothelium	107
10^6 nucleotides	T 1/2 ~ 2,1,6,3,1,1,>21D			--
50 mg/kg; 22 μg/24 hr	--	Rat	Urine	65
50 mg/kg; 2 ng/10 mg globin	--	Rat	Globin	65
?; 1 adduct/< 10^4 nucleotides	--	Mouse	Liver	182
Linear 0.08-0.6mM; 15-120 μmol N^7-methylguanine/mol DNA	--	Mouse	Lymphoid cells	79
Linear 0.08-1mM; 15-120 μmol N^7-methylguanine/mol DNA	--	Human	Cells	79
60 μg/ml 3 hr; 102, 14, 6.2 μmol/mol DNA	--	V79 cell culture	--	156
16 and 100 mg/kg; 0.8-7.0 μmol/mole guanine	T 1/2 = 5.5H	Mouse	Liver	53
16 and 100 mg/kg; 1.05-66 μmol/mole guanine	T 1/2 = 5.2H	Mouse	Kidney	53
0.87 mmol/kg; 242, 24 μmol/mol DNA	--	Rat	Kidney	53
		In vitro	N/A	80
		In vitro	N/A	131
--	--			
Binding to RNA, DNA, soluble protein	--	--	--	146

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
Nitroso-2,6-dimethyl	Direct acting	--	Radioisotope
Murpholine		--	""
N-Nitrosodi-n-propylamine	Direct acting	7-n-propyl guanine	
Nitroso(2-hydroxypropyl)(2-	Direct acting	--	""
oxopropyl)-amine	Direct acting	--	""
Nitrosomethylethylamine	Direct acting	--	""
Nitrosobis(2-oxyopropyl)amine	Direct acting	--	""
N-nitroso-bis-(2-oxopropyl)amine	Direct acting	N^7-methylguanine	
Z-ethyl-ONN-azoxymethane	Direct acting	--	""
Z-methyl-ONN-azoxymethane	Direct acting	--	""
Nitrosobis(2-hydroxypropyl)amine	Direct acting	--	""
N-nitrosomethylbenzylamine	Direct acting	N^7O^6-methylguanine	""
N-nitrosomethylbenzylamine	Direct acting	""	""
N-nitrosomethylbenzylamine	Direct acting	""	""
N-Nitrosomethylbenzyl amine	Direct acting	N^7-methylguanine	Radioisotope
N-Nitrosomethyl(4-methylbenzyl)amine	Direct acting	""	""

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
""	--	Rat, hamster	Liver	134
""	--	Rat, hamster	Liver	134
""	""	Rat	Liver	9
""	--	Rat, hamster	Liver	134
""	--	Rat, hamster	Liver	134
""	--	Rat, hamster	Liver	134
""	--	Rat, hamster	Liver	134
		Hamster	Liver, lung, pancreas	129
""	--	Rat, hamster	Liver	134
""	--	Rat, hamster	Liver	134
""	--	Rat, hamster	Liver	134
0.017mmol/kg; 344,46 μmol/mol guanine	--	Rat	Esophagus	94
0.017mmol/kg; 120,4.9 μmol/mol guanine	--	Rat	Liver	94
0.017mmol/kg; 65,7.7 μmol/mol guanine	--	Rat	Lung	94
0.017 mmol/kg; 10.3 μmol/mol DNA	--	Rat	Forestomach	94
0.017 mmol/kg; 22.4 μmol/mol DNA	--	Rat	Esophagus	94

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
""	Direct acting	""	""
""	Direct acting	""	""
N-Nitroso-N-methyl-N'-nitroguanidine	Direct acting	O^6,N^7-methylguanine	--
N-Nitroso-N-methylurethane	Direct acting	""	--
Nitrosopiperidine	Direct acting	Yes	--
Nitrosopyrroline	Direct acting	Yes	--
N-(2-oxopropyl)-N-nitrosourea	Direct acting	O^6,N^7-methylguanine,N^3-methyladenine	MS
N-Nitroso-2-oxopropylamine	Direct acting	O^6,N^7-methylguanine	UV absorbance
1-n-propyl-1-nitrosourea	Direct acting	O^6,N^7-n-propylguanine,O^6,N^7-iso-propylguanine	Radioisotope
β-proprolactone	Direct acting	O^6,N^7-alkylguanine	--
1,3-propane sultone	Direct acting	N^7,N^1,O^6-alkylguanine	UV absorbance
Propylene oxide	Direct acting	N^3-(2'-Hydroxypropyl)histidine	GC-MS
Streptozotocin	Direct acting	N^7,O^6-methylguanine, N^7,N^3-methyladenine	Radioisotope
""	Direct acting	""	""
""	Direct acting	""	""
""	Direct acting	""	""

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
0.017 mmol/kg; 30.8 µmol/mol DNA	--	Rat	Liver	94
0.017 mmol/kg; 13.6 µmol/mol DNA	--	Rat	Lung	94
--	--	Rodents	--	26
--	--	Rodents	--	26
--	--	Cultured, human cells	--	77
--	--	Cultured, human cells	--	77
Nonlinear, 10-40mM; ~25-60nmol/N^7-MeGua/mg DNA	--	In vitro	N/A	131
2.0mmol/kg; 55, 760 µmol/mol guanine	--	Rat	Liver	132
1mM, 68 pmol alkylation/mg DNA	--	In vitro	N/A	151
--	--	Rodents	--	26
--	--	In vitro	N/A	88
"Low" to 10 ppm; 0.9-13 nmol/g Hb	--	Human	Hemoglobin	
21 mg/kg; 486,21,16,35 pmol/µmol guanine	--	Rat	Liver	34
21 mg/kg; 289,11,13,17 pmol/µmol guanine	--	Rat	Kidney	34
21 mg/kg; 96,9,6,6 pmol/µmol guanine	--	Rat	Intestine	34
21 mg/kg; 5,<1,<1,<1 pmol/µmol guanine	--	Rat	Brain	34

COMPOUND	ACTIVE METABOLITES	ADDUCT STRUCTURE	MEASUREMENT TECH
""	Direct acting	""	""
""	Direct acting	5 adducts present	^{32}P-postlabeling
Vinyl chloride	Chloroethylene oxide and	3,N^4-ethenocytidine 1,N^6-ethenoadenosine, and	--
	chloroacetaldehyde	1,N^2-ethenoguanosine	
S-vinylhomosysteine	Same as ethionine		

DOSE/RESPONSE	PERSISTENCE	SPECIES	TISSUES	REF
21 mg/kg; 43,2,3,2 pmol/μmol guanine	--	Rat	Pancreas	34
?; 1 adduct/<10^4 nucleotides	--	Mouse	Liver	182
--	--	In vitro	N/A	192,196

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